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Drinking Water Criteria Document for Chlorobenzene (Draft)

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HEALTH EFFECTS CRITERIA DOCUMENT

FOR

CHLOROBENZENE

June 1985

CRITERIA AND STANDARDS DIVISION

OFFICE OF DRINKING WATER

U.S. ENVIRONMENTAL PROTECTION AGENCY

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16. ABSTRACT

The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on chlorobenzene. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of chlorobenzene
- Toxicokinetics and human exposure to chlorobenzene
- Health Effects of chlorobenzene in humans and animals
- Mechanisms of toxicity of chlorobenzene
- Quantification of toxicological effects of chlorobenzene

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* Prepared by the Science and Technology Branch

I. SUMMARY

Chlorobenzene (CB) has been used industrially in great quantities. It has been detected in raw water sources, waste waters and even in finished drinking water supplies.

Chlorobenzene is poorly soluble in water, but highly soluble in lipids. It is absorbed readily from the lungs, gastrointestinal tract and, to a lesser degree, through the skin. Chlorobenzene is metabolized *in vivo* principally to 4-chlorocatechol and 4-chlorophenol. The metabolites are eliminated from the body in bile (indirectly), feces, urine and expired air. The phenolic metabolites are excreted mainly as conjugates of glucuronic and sulphuric acids, and of glutathione as mercapturic acid.

The lower chlorinated benzenes, including chlorobenzene, are relatively resistant to biodegradation and are more so to chemical and physical degradation. This resistance, plus the compounds' ability to concentrate in fatty tissues, results in their tendency to accumulate in the environment.

Chlorobenzene has been shown to be toxic to various aquatic species, including fish and other aquatic organisms consumed by humans, microorganisms, insects, a variety of laboratory animals and humans. Acute, high dose effects of these compounds include sedation, anesthesia and death due to respiratory failure. Central nervous system (CNS) stimulation may precede CNS depression. Chronic exposure to these substances may result in blood dyscrasias and lung, liver and kidney damage. Porphyria due to effects on porphyrin synthesis often accompanies the liver damage.

No reports of reproductive and/or teratogenicity studies have been found in the peer-reviewed literature. Dow Chemical Company has completed teratology studies of chlorobenzene in rats and rabbits. In the Phase I studies, there was no evidence of teratological changes in the rats, but there were some suggestive changes in the rabbits at the highest dose tested. The experiments with the rabbits were repeated in the Phase II study. No teratogenic effects were seen during this experiment.

Chlorobenzene has been shown to cause mutagenic effects in one higher plant species and certain microorganisms. No reports were found in the open literature concerning the carcinogenic effects of chlorobenzene in the human or other animals. However, a draft technical report on the carcinogenesis bioassay of chlorobenzene (gavage studies in F344 rats and B6C3F1 mice) states that, under the conditions of the bioassay, chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in the high dose male rats, providing some but not clear evidence of carcinogenic activity in male rats (NTP, 1983). Following evaluation of these data, the EPA Carcinogen Assessment Group determined that it was inappropriate to derive a carcinogenic potency factor or estimated excess lifetime cancer risks. In addition, the EPA classified chlorobenzene on Group C: Possibly Carcinogenic to Humans. This classification is reserved for chemicals for which limited evidence of carcinogenicity in animals exists.

II. GENERAL INFORMATION AND PROPERTIES

Chemical and Physical Properties

At room temperature (20°C), chlorobenzene (CB) is a colorless, neutral liquid of molecular weight 112.6, with a distinctive odor of almonds. It is nearly insoluble in water (490 mg/l), but miscible in all proportions with many organic liquids, such as alcohol, benzene, the chlorinated solvents, ethers and aliphatic and aromatic hydrocarbons (Irish, 1963). At 25°C, CB has a specific gravity of 1.106 and a vapor pressure of 11.8 mm Hg. It solidifies at -45°C and boils at 132°C. It forms an azeotrope with water which boils at 90°C (71.6% chlorobenzene, 28.4% water, by weight). Since it is slightly heavier than water, it may sink to the bottom of still bodies of water.

In air, 1 ppm CB = approximately 4.6 mg/m^3 and 1 mg/l = 217 ppm at 25°C, 760 mm Hg (Irish, 1963).

Sato and Nakajima (1979) determined partition coefficients for CB. While the water/air coefficient was low (4.1), the oil/air coefficient was quite high (3763). The blood/air coefficient was 30.8. In addition, the oil/water coefficient was 918. Thus, chlorobenzene is quite lipophilic (log P = 2.96).

Additional evidence suggests a moderate potential for bicconcentration. Leo, et al. (1971) reported an octanol/water partition coefficient of about 700. Lu and Metcalf (1975) studied the environmental fate of and biodegradability of CB in a model ecosystem containing Daphnia, mosquito larvae, snails, fish and green algae. The data indicated that considerable ecological magnification (EM) occurred. EM values ranged from 645 for fish to 4200 for algae.

Human thresholds for detection of chlorobenzene have been reported. The odor and taste threshold for CB in water has been reported to range from 0.41-1.5 ug/l (Tarkhova, 1965) to 10-20 ug/l (Varshavskaya, 1968). A.D. Little (1968), in a report to the Manufacturing Chemists Association, listed the odor threshold as 0.21 mg/l (medium not designated). None of these citations described the experimental conditions/protocols used in the determination of these thresholds. Therefore, it is difficult to interpret these data, judge their validity or account for the disparity in the findings of the investigators.

Production and Use

Chlorobenzene is used in significant quantities in this country. U.S. production in 1977 was about 150,000 kkg (the most recent data available to the Office of Drinking Water) (USTIC, 1978). Imports accounted for nearly 500 kkg. At that time, approximately 35% of the chlorobenzene was utilized in the production of chloronitrobenzenes, 10% in diphenyl oxide production, 10% for rubber intermediates and 7% for DDT. About 30% of the annual production volume was used as a solvent in adhesives, paints, waxes, and polishes and as an inert process solvent.

III. HUMAN EXPOSURE

Humans may be exposed to chlorobenzene in drinking water, food, and air. Detailed information concerning the occurrence of and exposure to chlorobenzene in the environment is presented in another document entitled "Occurrence of Chlorobenzene in Drinking Water, Food, and Air" (Letkiewicz et al. 1983). This section summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food, and air.

Exposure Estimation

This analysis is limited to drinking water, food, and air, since these media are considered to be general sources common to all individuals. Some individuals may be exposed to chlorobenzene from sources other than the three considered here, notably in occupational settings and from the use of consumer products containing chlorobenzene. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and several factors over which there is little control. Where one lives, works, and travels, what one eats, and physiologic characteristics related to age, sex, and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Unfortunately, data and methods to estimate exposure of identifiable population subgroups from all sources simultaneously have not yet been developed. To the extent possible, estimates are provided of the number of individuals exposed to each medium at various chlorobenzene concentrations. The 70-kg male is used for estimating intake.

a. Water

Cumulative estimates of the U.S. populations exposed to various chlorobenzene levels in drinking water from public water systems are presented in Table IV-I. The values in the table were obtained using Federal Reporting Data Systems data on populations served by primary water supply systems (FRDS 1983) and the estimated number of these water systems that contain a given

Table IV-I. Total Estimated Cumulative Population (in Thousands) Exposed to Chlorobenzene in Drinking Water Exceeding the Indicated Concentration

System type	Number of people served in U.S. (thousands)	Cumulative population (thousands) exposed to concentrations (ug/l) of: ≥0.5	>5
Groundwater	73,473	177	0
Surface water	140,946	0	0
Total	214,419	177	0
(% of total)	(100%)	(0.1%)	(0.0%)

level of chlorobenzene. An estimated 177,000 individuals (0.1% of the population of 214,419,000 using public water supplies) are exposed to levels of chlorobenzene in drinking water at or above 0.5 ug/l. It is estimated that no individuals are exposed to levels greater than 5 ug/l. All exposure to chlorobenzene in drinking water at levels above 0.5 ug/l is expected to be from groundwater sources.

No data were obtained on regional variations in the concentration of chlorobenzene in drinking water. The highest concentrations are expected to occur near sites of its use in pesticide production and degreasing operations.

Daily intake levels of chlorobenzene from drinking water were estimated using various exposure levels and the assumptions presented in Table IV-II. The data in the table suggest that the majority of the persons using public drinking water supplies would be exposed to intake levels below 0.014 ug/kg/day.

Table IV-II. Estimated Drinking Water Intake of Chlorobenzene

Exposure level (ug/l)	Persons using supplies exposed to indicated levels: Population	% of Total Population	Intake (ug/kg/day)
≥ 0.5	177,000	0.1%	≥0.014
> 5.0	0	0.0%	>0.14

Assumptions: 70-kg man, 2 liters of water/day.

An indication of the overall exposure of the total population to chlorobenzene can be obtained through the calculation of population-concentration values. These values are a summation of the individual levels of chlorobenzene to which each member of the population is exposed. An explanation of the derivation of these values is presented in Appendix C. The population-concentration estimates were 8.9×10^4 ug/l x persons (best case) and 1.1×10^8 ug/l x persons (worst case). Midrange estimates could not be obtained due to the absence of positive values of chlorobenzene in surface water.

Assuming a consumption rate of 2 liters of water/day, population-exposure values of 1.8×10^5 ug/day x persons (best case) and 2.2×10^8 ug/day x persons (worst case) were derived.

b. Diet

No information was obtained in levels of chlorobenzene in foods, other than mother's milk, in the United States. Therefore, no estimate of the daily intake of chlorobenzene from the diet could be made.

c. Air

Exposure to chlorobenzene in the atmosphere varies from one location to another. The highest level of chlorobenzene reported in the atmosphere was 97,000 ng/m^3 (97 ug/m^3) (Singh et al. 1979 cited in Brodzinsky and Singh 1982). High levels, averaging greater than 10,000 ng/m^3 (10 ug/m^3), have been detected in other areas. Normal levels, however, are somewhat lower. Brodzinsky and Singh (1982) calculated median air levels of chlorobenzene for rural/remote areas, urban/suburban areas, and source dominated areas of 0.0 ng/m^3 (0.0 ug/m^3), 1,500 ng/m^3 (1.5 ug/m^3), and 140 ng/m^3 (0.14 ug/m^3), respectively.

The monitoring data available are not sufficient to determine regional variations in exposure levels for chlorobenzene. However, urban and industrial areas appear to contain higher levels, as expected.

The daily respiratory intake of chlorobenzene from air was estimated using the assumptions presented in Table IV-III and the median and maximum levels for chlorobenzene reported above. The estimates in Table IV-III indicate that the daily chlorobenzene intake from air for adults in urban/suburban

Table IV-III. Estimated Respiratory Intake of Chlorobenzene

Exposure (ug/m^3)	Intake (ug/kg/day)
Rural/remote (0.0)	0.0
Source dominated (0.14)[a]	0.046
Urban/suburban (1.5)	0.49
Maximum (97)	32

[a]Value reported for source dominated areas was lower than that reported for urban/suburban areas.

Assumptions: 70-kg man, 23 m^3 of air inhaled/day (ICRP 1975).

areas is approximately 0.5 ug/kg/day. In contrast, the intake calculated using the maximum level reported is 32 ug/kg/day; few if any persons are believed to be exposed at that level. The values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

In addition to the available monitoring data, Systems Applications, Inc. (1982) has provided estimates of atmospheric levels of chlorobenzene by applying air dispersion models to chlorobenzene emission sources. The computed average concentrations of chlorobenzene and the number of individuals estimated to be exposed to these concentrations are presented in Table IV-IV. Specific point sources in the table are individually identified sources with known locations and modes and rates of emissions. These are generally manufacturing plants. General point sources are sources which are numerous, small, or of too uncertain location to be treated individually. However, these sources produce isolated patterns of significant concentration. Area sources are sources that are numerous and emit only small concentrations of the chemical (e.g., home chimneys, automobiles). The Systems Applications estimates for chlorobenzene suggest that a relatively small number of individuals (approximately 1.5 million) are exposed to atmospheric chlorobenzene concentrations of greater than 2.5 ug/m^3 (2,500 ng/m^3).

Table IV-IV also presents a total population-concentration estimate of 1.41×10^8 ug/m^3 x persons. Assuming an inhalation rate of 23 m^3 of air/day, a population-exposure of 3.24×10^9 ug/day x persons was calculated.

Table IV-IV. Exposure and Dosage Summary for Airborne Chlorobenzene

Concentration level (ug/m^3)	Population exposed (persons)				Dosage (ug/m^3 x persons)			
	Specific point source	General point source	Area source	U.S. total	Specific point source	General point source	Area source	U.S. total
100	0	2,200	0	2,200	0	300,000	0	300,000
50	8	14,000	0	14,008	493	1,100,000	0	1,100,493
25	60	56,000	0	56,060	2,130	2,540,000	0	2,542,130
10	357	215,000	0	215,357	6,210	4,840,000	0	4,846,210
5	1,506	485,000	0	486,506	14,000	6,700,000	0	6,714,000
2.5	5,049	1,490,000	0	1,495,049	26,000	10,100,000	0	10,126,000
1	14,038	--	0	--	40,800	14,200,000	0	14,240,800
0.5	56,813	--	0	--	69,800	17,700,000	0	17,769,800
0.25	186,820	--	0	--	114,000	22,700,000	0	22,814,000
0.1	615,266	--	0	--	180,000	32,500,000	0	32,680,000
0.05	1,223,656	--	0	--	223,000	41,300,000	0	41,523,000
0.025	--	--	0	--	--	49,300,000	0	--
0.01	--	--	0	--	--	57,800,000	0	--
0.001	--	--	505,140	--	--	--	1,082	--
0.0005	--	--	9,149,730	--	--	--	8,281	--
0.00025	--	--	27,819,254	--	--	--	14,972	--
0.0001	--	--	95,892,857	--	--	--	25,169	--
0.00005	--	--	140,098,202	--	--	--	28,469	--
0	4,065,115	--	158,679,135	--	258,000	141,000,000	29,100	141,287,100

Note: The use of "--" as an entry indicates that the incremental increase in the dosage or the population exposed is not significant (relative to the last entry in that column or to an entry in another column at the same row) or that the exposure of the same population may be counted in another column.

Source: Systems Applications 1982

SUMMARY

Table IV-V presents a general view of the total amount of chlorobenzene received by an adult male from air and drinking water. Four separate exposure levels in air and three exposure levels in drinking water are shown in the table. Insufficient data were obtained on levels of chlorobenzene in foods to assess the contribution of chlorobenzene in the diet to total chlorobenzene exposure.

The data presented have been selected from an infinite number of possible combinations of concentrations for the two sources. The actual exposures encountered would represent some finite subset of this infinite series of combinations. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to chlorobenzene at any of the combined exposure levels. The data presented represent possible exposures based on the occurrence data and the estimated intakes.

Brodzinsky and Singh (1982) calculated a median urban/suburban air level of chlorobenzene of 1.5 ug/m^3 based on air monitoring data. Assuming an air level of 1.5 ug/m^3, drinking water would be the predominant source of chlorobenzene exposure in the adult male at drinking water levels above 17 ug/l. An accurate assessment of the number of individuals for which drinking water is the predominant source of exposure cannot be determined from the data since specific locations containing high concentrations of chlorobenzene in drinking water and low concentrations of chlorobenzene in ambient air and food are unknown.

Population-exposure estimates for chlorobenzene in drinking water and air were presented previously. Estimates for drinking water ranged from 0.0018-2.2 x 10^8 ug/day x persons; the estimate for ambient air was 3.24 x 10^9 ug/day x persons. These estimates suggest that ambient air may be a greater source of exposure to chlorobenzene than drinking water on a general population basis. Comparison of these estimates, however, may be deceiving since the same population-exposure level can occur if: 1) a whole population is exposed to moderate levels of a chemical or 2) some segments of the same population are exposed to high levels and others to low levels. The population-intake values presented give no indication of the relative predominance of drinking water and air as specific sources of chlorobenzene on a site-by-site or sub-population basis.

The relative source contribution data are based on estimated intake and do not account for a possible differential absorption rate for chlorobenzene by route of exposure. The relative dose received may vary from the relative intake. In addition, the relative effects of the chemical on the body may vary by different routes of exposure.

Table IV-V. Estimated Intake of Chlorobenzene from the Environment by Adult Males in ug/kg/day (% from Drinking Water)

Concentration in drinking water (ug/l)	Concentration in air			
	Rural/remote (0.0 ug/m^3)	Source dominated (0.14 ug/m^3)	Urban/suburban (1.5 ug/m^3)	Maximum (97 ug/m^3)
0	0.0 (--)	0.046 (0%)	0.49 (0%)	32 (0%)
0.5[a]	0.014 (100%)	0.060 (23%)	0.50 (2.8%)	32 (0.04%)
5.0[b]	0.14 (100%)	0.19 (74%)	0.63 (22%)	32 (0.4%)

Intake from each source (see Sections 5.1-5.3):

Water: 0.5 ug/l: 0.014 ug/kg/day
5.0 ug/l: 0.14 ug/kg/day

Air: 0.0 ug/m^3: 0.0 ug/kg/day
0.14 ug/m^3: 0.046 ug/kg/day
1.5 ug/m^3: 0.49 ug/kg/day
97 ug/m^3: 32 ug/kg/day

Food: Not included

[a] 177,000 individuals using public drinking water systems are estimated to be exposed to levels $\geq$ 0.5 ug/l (0.1% of population using public water supplies).

[b] No individuals using public drinking water systems are estimated to be exposed to levels > 5.0 ug/l.

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IV. KINETICS

Absorption/Retention

Although there apparently have been no studies of the systemic absorption of chlorobenzene, it should be well absorbed since it is highly lipid soluble. Because of this property, it can readily traverse many tissue barriers *in vivo*, such as the placenta and blood:brain barrier. It is absorbed from the lungs, the gastrointestinal tract and through the skin.

No data are available which demonstrate the percentage of a dose absorbed following exposure to CB in any medium. Based upon what is known about the absorption characteristics of benzene and the smaller chlorinated aliphatics (ethanes and ethylenes), it will be assumed that 100% of any oral dose is absorbed, while 60% of a dose inhaled over a period of one to several hours is absorbed and retained (Astrand, 1975;Dallas, et al., 1983).

Distribution

A distribution study has been done in male Sprague-Dawley rats following inhalation exposure (Sullivan, et al., 1983). Groups of 15 animals each were exposed at 100, 400 or 700 ppm ^{14}C-chlorobenzene (460, 1840 or 3220 mg/m^3, respectively) for 8 hours. Six of each group were exposed for 5 consecutive days. Another 3 were exposed for the first four days of the multiple exposure regimen, but then killed on the morning of the fifth day, 16 hours after their last exposure. The remaining 6 rats were exposed only on the fifth day for 8 hours. Liver, lung, kidney, blood, epididymal and perirenal fat were analyzed for

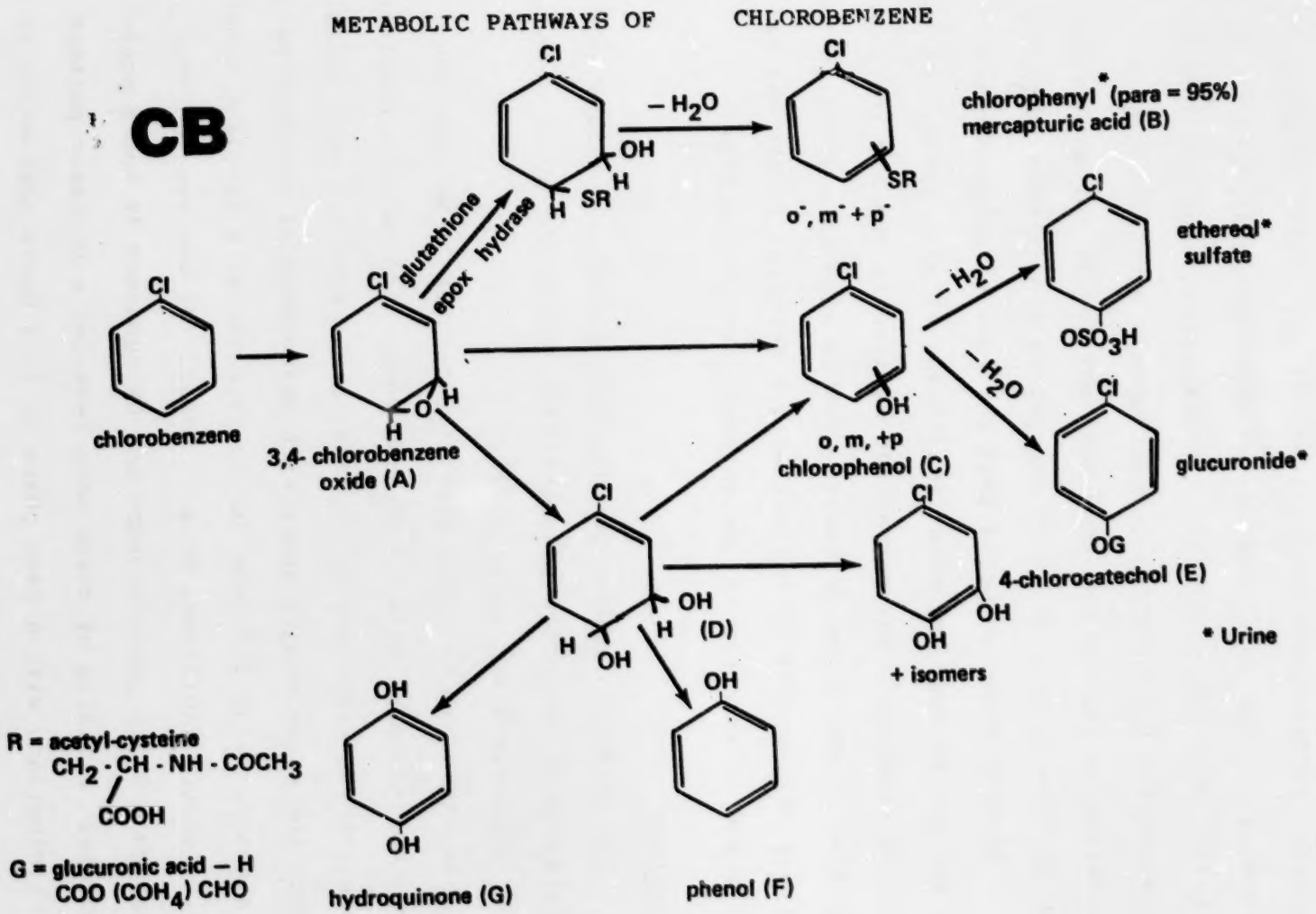
^{14}C content. As might be expected, the highest tissue burdens were found in the fat (epididymal and perirenal). Kidneys and liver also showed significant amounts. The amounts found in these tissues were proportional to dose except for the adipose tissue which showed greatly exaggerated accumulation with dose when compared to the other tissues. The ^{14}C burden of adipose tissue increased 8-10 fold when the exposure concentration increased from 100 to 400 ppm and 3-5 fold when the exposure increased from 400 to 700 ppm. There also was a tendency for multiply-exposed rats to exhibit higher tissue burdens that rats exposed only once.

Metabolism

Figure IV-1 depicts the metabolites of CB identified and the metabolic pathways proposed. The figure reflects a composite constructed from several studies (Spencer and Williams, 1950; Azouz, et al., 1953; Williams, 1959).

After a single 0.5 mg/kg oral dose of CB administered to rabbits, 27% was excreted unchanged in expired air within 1-2 days (Spencer and Williams, 1950; Azouz, et al., 1953). Some was excreted unchanged in the urine. The remaining portion was metabolized in vivo. Following conversion to a 3,4-arene oxide (A), CB was further metabolized via glutathione conjugation to chlorophenyl-mercapturic acid (B), via hydroxylation to o- and/or p-chlorophenol (C), or to p-chlorocatechol (E) from 3,4-dihydro-3,4-dihydroxychlorobenzene (D). Small amounts of phenol (F), hydroquinone (G) and some diphenols have been detected in the urine of some species.

Figure IV-1


METABOLIC PATHWAYS OF CHLOROBENZENE
CB
chlorobenzene
3,4- chlorobenzene oxide (A)
glutathione epox hydrase
$-H_2O$
chlorophenyl* (para = 95%) mercapturic acid (B)
o⁻, m⁻ + p⁻
o, m, +p chlorophenol (C)
ethereal* sulfate
OSO_3H
glucuronide*
OG
4-chlorocatechol (E)
+ isomers
* Urine
(D)
hydroquinone (G)
phenol (F)
R = acetyl-cysteine
$CH_2 \cdot CH \cdot NH \cdot COCH_3$
COOH
G = glucuronic acid – H
COO (COH_4) CHO

The metabolism of ^{14}C-chlorobenzene in thirteen mammalian species (as described by Williams, et al., 1975) is shown in Table IV-1. The basic pattern of metabolites appears to be similar, although quantitatively different, from one species to another. For example, p-chlorophenyl mercapturic acid formation is high in the hedgehog, but low in the guinea pig. Man appears to be similar to the guinea pig in this respect.

It also might be noted that the fate of chlorobenzene in the rat is dose dependent (Sullivan, et al., 1983). As the dose is increased, the percentage expired in the air increases and that in the urine decreases. There also is a shift in the urinary metabolites in the rat with a decrease in the mercapturic acids and an increase in the sulfides and glucuronides.

Excretion

The principal routes of excretion for chlorobenzene are exhalation as the unchanged substance and in the urine as metabolites (see Figure IV-1 and Table IV-1).

As stated above, the ratio of unchanged to metabolized compound increases with increasing dose. The rate of respiratory excretion was evaluated by expressing the data for each animal within the experimental group as a percentage of the respiratory elimination total remaining to be excreted at a specific time(s) post-exposure (Sullivan, et al., 1983). It was assumed that 100% remained 0 hours post-exposure and 0% remained 48 hours post-exposure. Plotting of these data revealed a biphasic pattern of elimination, with a fast phase up to 4 hours post-exposure and a slow phase over the remaining periods (sampled at 12, 24, 36 and

Table IV-1

Metabolism of ^{14}C-Chlorobenzene in Different Species

(Williams, et al., 1975)

% of 24-hour excretion of ^{14}C as:

Species	p-Chlorophenol	p-Chlorocatechol	p-Chlorophenyl-mercapturic acid
Man	33	31	19
Rhesus monkey	19	37	40
Squirrel monkey	14	37	50
Capuchin monkey	19	36	41
Dog	14	45	42
Ferret	33	31	24
Hedgehog	20	12	65
Rabbit	29	38	26
Rat	23	22	49
Mouse	20	31	42
Gerbil	13	26	51
Hamster	15	23	43
Guinea pig	27	35	21

48 hours post-exposure)(see Table IV-2). The exposure regimen effect was significant only at the 700 ppm concentration, with the multiply-exposed rats showing a shorter fast phase $t_{1/2}$ than singlyexposed rats. At this concentration, also, the fast phase increased in length while the short phase decreased in length for animals exposed only once or over the five days. This blurring of the distinction between the fast and slow phases indicates saturation of the metabolic clearance pathways.

Table IV-2

Apparent Half-lives of Respiratory Elimination Following Inhalation Exposure of Rats to ^{14}C-Chlorobenzene

(Sullivan, et al., 1983)

Exposure Concentration (ppm)	Exposure Regimen[a]	Apparent $t_{1/2}$ (hr)[b]		r^2
		Fast phase	Slow phase	
100	S	0.81	8.77	0.96
100	M	0.60	9.24	0.98
400	S	1.13	7.62	0.99
400	M	0.83	8.56	0.99
400[c]	S	1.19	7.70	0.98
700	S	3.65[d]	5.73[d]	0.99
700	M	1.62[d,e]	6.60[d,e]	0.99

a S = single exposure, M = multiple exposure
b Half-lives ($t_{1/2}$) were calculated from the rate constants of a biphasic log-linear regression
c Data from the repeat 400 ppm exposure block
d Significantly different from the 100- and 400-ppm groups ($p<0.05$)
e Significantly different from single exposure ($p<0.05$)

V. HEALTH EFFECTS IN ANIMALS

Chlorobenzene produces sedation and anesthesia after acute oral or parenteral administration. Acute poisoning is characterized by signs of disturbance of the central nervous system (CNS). There may be hyperexcitability, restlessness, muscle spasms or tremors followed by varying degrees of CNS depression. The most frequent cause of death is respiratory failure. Acute exposure at very high levels also may result in liver and kidney damage as well as effects on porphyrin synthesis.

Several investigators have determined acute LD_{50}s and LC_{50}s after oral and inhalation exposure to MCB in several species. These data are presented in Table V-1.

In rats, the subcutaneous injection of a 4-5 g/kg body weight dose of chlorobenzene caused no immediate effects, but resulted in death in a few days. On autopsy, the rats were found to have necrosis in the liver and kidneys. Larger doses produced CNS depression and death in a relatively short period of time. Between 7 and 8 g/kg were fatal in a few hours (cited in von Oettingen, 1955).

Rozenbaum, et al. (1947) conducted a study of the acute toxicity of CB. In mice receiving 5-30 mg/l CB by inhalation over a two-hour period, 65% died, with the onset of narcosis being earlier and the intensity stronger than for comparable doses of benzene. All animals exposed to an atmosphere of 20 mg/l or higher exhibited clonic and tonic spasms and died of respiratory failure. Six rabbits received a series of 2-20 doses of 0.09 mg CB/kg over a two-week period. One animal died after six injections;

Table V-1

Acute Toxicity Data for Chlorobenzene

Animal	Route	LD_{50}	LC_{50}	Reference
Rat	Oral	2.91 g/kg		Irish, 1963
	Oral	2.39 g/l		Varshavskaya, 1967
Rat	Oral	3.4 g/kg		Vecerek, et al., 1976
Rabbit	Oral	2.83 g/kg		Irish, 1963
	Oral	2.25 g/l		Varshavskaya, 1967
Guinea pig	Oral	5.06 g/l		Varshavskaya, 1967
Mouse	Oral	1.44 g/l		Varshavskaya, 1967
Mouse	Inhalation	(2 hours)	20 mg/l	Rozenbaum, et al., 1947
	Inhalation		8.2-9.1 mg/l	Bonnet, et al. 1979
Guinea pig	Inhalation		0.05 mg/l	Lecca-Radu, 1959

two died after eight injections. Blood dyscrasias consisted of an increase in the number of stabnuclear forms and pseudoeosinophils, while no regular reduction in the number of leukocytes was detected. Kidneys showed a swelling of the tubular and glomerular epithelia. The liver exhibited swelling and deposition of fatty droplets.

In addition to producing hepatic and renal toxicity, chlorobenzene also alters pancreatic function (Yang, et al., 1979). A single intraperitoneal dose of chlorobenzene in sesame oil (5 mmol/kg; 560 mg/kg) was administered to adult male Holtzman rats. Control animals received an equivalent volume of sesame oil (1 ml/kg). Experimental measurements were made 24 hours later. Bile duct-pancreatic flow (BDPF) was increased 10-fold over that observed in control animals ($P<0.05$). Protein concentration of the effluent was reduced by more

than two-thirds, from 21.7 mg/ml to 6.2 mg/l ($P<0.05$). The rate of bile flow also was increased significantly ($P<0.05$). Bile chloride levels and serum GPT activity were not altered. The mechanism by which these changes occur is not known, but it appears not to involve secretin or cholinergic stimulation of the pancreas, nor does it occur secondarily to liver damage. The significance of these findings remains unknown.

Ariyoshi, et al. (1975a) administered 250 mg CB/kg orally to 6 rats for 3 days. Animals were sacrificed 24 hours after the final dose. An increase in Δ-aminolevulinic acid (Δ-ALA) synthetase in liver homogenates was observed when compared with controls.

Rimington and Ziegler (1963) gavaged rats with each of seven chlorinated benzenes, including CB. The chemical (in concentrations of 100-200 mg/l) was suspended in either 1 percent cellofas, liquid paraffin or propylene glycol. Doses were increased gradually to the point of causation of high porphyrin excretion, but minimum fatality. When 3 rats were given 1140 mg CB/kg/day for 5 days, increases in urinary coproporphyrin III, uroporphyrin, porphobilinogen (PBG) and Δ-ALA were observed when compared with controls. Severe liver damage with extensive necrosis and fatty changes occurred. There was also an increase in protoporphyrin and uroporphyrin levels in the liver. When compared with the higher substituted chlorinated benzenes, CB was less potent, requiring a higher dose to affect a similar response.

Cameron, et al. (1937) concluded that chlorobenzene, given subcutaneously or intravenously to an unspecified number of rabbits, caused leukopenia. Three successive daily doses of 1 cc (1130 mg) caused the white cell count to fall from 7500 cells/mm^3 to 1850 cells/mm^3 within a week in a single animal with a gradual return to normal. In contrast, Rozenbaum, et al. (1947) reported an increase in red and white cell count after repeated daily subcutaneous administration of chlorobenzene (0.9 mg/kg for up to 20 doses) to rabbits.

Zub (1979) reported leukopenia as well as lymphocytosis and fatty degeneration of the liver in Swiss mice (five males and five females per group) exposed via inhalation to CB 7 hours/day for three weeks at 2.5 mg/l (2500 mg/m^3) or for three months at 0.1 mg/l (100 mg/m^3). Only half of the animals exposed to 2.5 mg/l survived until the end of the experiment. In the survivors, loss of appetite, emaciation and marked somnolence were observed, along with body weight loss. Fatty degeneration was observed in the liver leading to yellow atrophy. White blood cell counts fell significantly in 4 of the 5 survivors. One mouse exhibited leucocytosis which the author attributed to a "neoplastic tendency." The percentage increase in lymphocyte count was offset by a corresponding decrease in the percentage of neutrophils. Animals exposed for three months at 0.1 mg/l averaged white cell counts of 5210 cells/mm^3 as compared with controls averaging 7670 cells/mm^3 ($P<0.001$). Control blood smears showed 60% lymphocytes, while treated smears showed 80%. Neutrophils were exactly the reverse: 39% in the controls and 20% in the treated. Basophils numbered 0.8% in the treated group.

Khanin (1969) exposed rats to 0.1 or 1.0 mg/m^3 continuously for 70 to 82 days. CNS depression was observed as well as toxic encephalopathy and inflammation of internal organs. Histologic foci of large cell hyperplasia in the kidneys and protein dystrophy of the liver also were observed.

Knapp, et al. (1971) reported the results of subchronic toxicity studies with CB in beagle dogs and rats. These studies were conducted by Hazelton Laboratories for the Monsanto Company (Hazelton, 1967a,b). Daily oral doses of 0, 27.25, 54.5 or 272.5 mg/kg/day in gelatin capsules were given to dogs of both sexes five days per week over a 93-day period. There were four males and four females in each dose group. After 14 to 21 doses of 272.5 mg/kg, four of the eight dogs died (2 males and 2 females). These animals showed immature leukocytes, lowered blood sugar levels and elevated serum glutamic pyruvic transaminase and alkaline phosphatase activities. In some dogs, an increase occurred in total bilirubin and total cholesterol. The elevated enzyme levels, bilirubin and cholesterol reflect the liver damage that was confirmed upon microscopic examination. The presence of immature leukocytes indicates that chlorobenzene affected the bone marrow at the high dose. Also, at the high dose, gross and microscopic pathology was evident in the liver, gastrointestinal mucosa and hematopoietic tissue of the dogs that died, and to a lesser extent, in the dogs sacrificed after 65 or 66 daily doses. Signs of toxicity were not consistent in dogs receiving the intermediate dose. One female had four episodes of diarrhea and one of vomiting. One male showed conjunctivitis throughout

the study and had one episode of emesis. Three dogs gained weight; three lost weight and two maintained their weights. As a group, there was no statistically significant change from control. One male had an increased kidney/body weight ratio; one female had a slightly increased liver:body weight ratio. No statistically significant changes occurred in the group as a whole. No adverse effects were apparent in the low dose group. change seen.

Rats were given 0, 12.5, 50, 100 or 250 mg CB/kg in corn oil by gavage for 93-99 consecutive days (Hazleton, 1967b). Male rats showed retarded growth at the highest dose level. At the intermediate and high dose levels, an increase in liver and kidney weights was observed in some animals. However, the group mean values were not significantly different from control. No significant histopathological changes were noted. No adverse effects were noted in the low dose group.

Rats, rabbits and guinea pigs were exposed by inhalation seven hours a day, five days a week, for a total of 32 exposures over a period of 44 days to concentrations of 200, 475 or 1000 ppm (Irish, 1963). The response of the animals in the high dose group was characterized by "histopathological changes" in the lungs, kidneys and liver. In the middle dose group, there was an increase in liver weight and a slight liver "histopathology." In the low dose group, no adverse effects were observed. In none of the groups was a hematological change seen (parameters studied were not identified).

CB was administered orally to rats five days a week for a total of 137 doses over 192 days, in dose groups of 14.4, 144 or 288 mg/kg (Irish, 1963). In the middle and high dose groups, there were significant increases in liver and kidney weight and some "histopathological changes" in the liver. No significant changes were observed in the low dose group. Blood and bone marrow were normal in all animals.

Industrial Bio-Test (IBT) conducted a 90-day inhalation toxicity study for Monsanto using 4 groups of beagle dogs (8 dogs per group: 4 males, 4 females), and 4 groups of albino rats (30 rats per group: 15 males, 15 females) (IBT, 1979). Animals of both species were exposed to 0, 0.75, 1.50 or 2.00 mg/L air (0, 104 ppm, 205 ppm or 273 ppm) 6 hours per day, 5 days per week, for a total of 62 exposures (12.4 weeks). It is unknown if this study has been validated. Five of the eight dogs in the high dose group and two of the eight dogs in the intermediate dose group were sacrificed before the conclusion of the test period (on the average, 30th day) because they were in a moribund condition. Weight gains for the high dose dogs were significantly lower than for the corresponding controls. These animals had lower total leukocyte counts than the controls and elevated values of serum alkaline phosphatase, glutamine pyruvic transaminase and glutamate oxaloacetate transaminase. The high dose females showed dogs significantly higher absolute liver weights;the mid-dose males exhibited siginficantly lower absolute heart weights ($p<0.05$). Both mid- and high dose females had significantly higher absolute organ weights for the pancreas

($p<0.05$). Gross and microscopic histologic changes for the seven dogs in moribund condition included enlarged and hardened livers and, in the high dose animals, vacuolization of the hepatocytes. The bone marrow of 2 males and 3 females in the high dose group was aplastic; the fourth female in this group exhibited marrow which was hypoplastic. Also, in this high dose group, 1 male and 3 females showed kidney pathology characterized by cytoplasmic vacuolization of the epithelium of the collecting tubules. Two males had bilateral atrophy of the seminiferous epithelium of the testes. Weight gains for the intermediate dose dogs were slightly less than controls. No gross or microscopic abnormalities were observed in any of the surviving intermediate dose dogs or in any of the low dose dogs.

Among the rats, no gross or microscopic pathological changes were reported in any of the low- and intermediate dose rats. Significantly higher organ-to-body weight ratios for liver and kidneys and higher absolute ovary weights were observed in the high dose females. High dose males exhibited significantly higher absolute kidney weights. Low- and mid-dose males had higher absolute weight as well as organ to body and organ:brain weight ratio for the lung than controls and lower results for each parameter in the pancreas.

A 6-month inhalation study in male and female beagle dogs was conducted by Monsanto (1980). The parameters of this study were essentially the same as the aforementioned subchronic study by IBT. Adolescent dogs of both sexes (6 dogs/sex/group) were subjected to 123 6-hour exposures over 6 months at target

levels of 0, 0.78, 1.57 or 2.08 mg/l. Actual levels were within ± 10% of the target levels.

Each animal was weighed and examined weekly. Following the last exposure, all animals were examined, sacrificed and necropsied. Over 20 tissues were excised, fixed and prepared for histological examination. The adrenals, brain, heart, kidney, liver, pituitary and testes were weighed. Clinical chemistry evaluation of blood and urine specimens was done twice before the experiment began, twice during the first four weeks of exposure, once a month over the next four months and at sacrifice. Bone marrow samples aspirated from the pelvic ileum were examined from all animals before exposure and at sacrifice. Marrow was examined from 2 dogs/sex/group at monthly intervals during the experiment. Determination of the myeloid: erythroid precursor cell ratio was made at each time point.

No statistical differences in mean weekly body weights or food consumption between controls and treated groups were observed during the course of the entire study. Emesis, abnormal stools and eye irritation were observed during the course of the experiment, beginning at week 7. No statistical association between exposure and eye irritation was observed. There was a dose-related, sex-independent increased occurrence of emesis, both in terms of the number of animals affected and the persistence of the sign. Treated animals exhibited this sign during weeks 7-10 and then again during weeks 17-27, while controls exhibited emesis sparingly during weeks 18-24. A significant increase in the frequency of abnormal stools was observed

among treated females during weeks 7-22 ($P<0.001$).

There was a statistically-significant reduction in the absolute adrenal weights of males in the middle and high dose groups ($P<0.05$). A similar dose-trend relationship, though not statistically-significant, was observed in the females. There was no statistically significant interpretative abnormality detected in bone marrow at any dose in either sex.

The investigating laboratory determined that "the various enzyme changes considered to be reflective of liver abnormalities occurred with no consistency that could be linked to individual dogs showing marked emesis and abnormal stools. Sex-independent increases ($P<0.01$) of alkaline phosphatase were mild and not outside of the normal range for beagles. There were no macroscopic or microscopic lesions evident in dogs that could be attributed to the treatment regimen. Therefore, "no toxicological significance can be demonstrated for the observed statistically-significant ($P<0.05$) increases of liver-to-body weight ratio on middle and high exposure group female dogs."

Monsanto concluded that, except for the clinical observations of emesis and abnormal stools and the sporadic, statistically-significant increases in alkaline phosphatase, there were no dose-related effects observed at any dose level. A more conservative interpretation of the data would lead to the conclusion that only the lowest dose was without significant effect.

Dilley (1977), in a study performed for NIOSH, exposed groups of 32 male rats and 32 male rabbits to 0, 75 or 250 ppm

chlorobenzene vapor 7 hours/day, 5 days/week for up to 120 exposures (24 weeks). At 5, 11 and 24 weeks of treatment, groups of animals were sacrificed. Hematological, clinical chemical, gross and microscopic histological changes were examined.

For the rats, the data showed that, at 11 weeks, there were increased absolute liver and heart weights and increased organ:body weight ratios for liver in the low dose rats. This group also showed increased liver:brain, heart:brain and kidney:brain ratios. The high dose rats exhibited only increased liver:body weight ratio. In animals from both dose groups, there were occasional focal lesions in the adrenal glands, focal lesions in the kidney tubules and liver and kidney congestion which appeared to be treatment-related. No chemical-related pathology was evident at 24 weeks.

At 11 weeks, in the rats at both doses, there was an increase in the number of blood platelets and reticulocytes and a decrease in microcellular volume with an increase in micro-hematocrit. In the high dose group, the white cell count was increased along with the percentage of polymorphonuclear leukocytes. At 24 weeks, the only significant difference seen was an increase in the percentage of reticulocytes in the high dose group ($p<0.01$).

SGOT activity was decreased in the high dose rats at all three sacrifice times.

In the rabbits, dose-related renal congestion was apparent at 5 weeks, some renal congestion at 11 weeks, but no treatment-

related changes were seen at 24 weeks. In addition, increased liver and lung weights were noted in the high dose group at 24 weeks. While the white blood cell count was significantly increased at 5 weeks in the high dose animals, this difference was not observed at the 11 and 24 week sacrifice. However, at 24 weeks, the numbers of polymorphonuclear leukocytes were increased significantly ($p<0.05$). SGOT activity was decreased significantly in the high dose animals at all sacrifice times. Other significant changes seen at 5 weeks in one or both dose groups did not persist after 24 weeks.

Varshavskaya (1968) studied the chronic effects of CB on white rats. The rats were treated daily with solutions of CB in oral doses of 0.1, 0.01 or 0.001 mg/kg. Controls received the vehicle, sunflower oil, alone. The experiments lasted nine months. At the 0.1 mg/kg dose, effects (not specified) of the greatest magnitude on the nervous system were observed. Lesser effects were observed at 0.01 mg/kg and no adverse effects were seen at 0.001 mg/kg. CB, at 0.1 mg/kg, caused a statistically-significant inhibition of erythropoiesis, thrombocytosis, inhibition of mitotic activity in the bone marrow and eosinophilia. Similar, but less pronounced effects were observed at 0.01 mg/kg, whereas 0.001 mg/kg had no effect. At doses of 0.1 and 0.01 mg/kg, MCB caused a significant increase in blood alkaline phosphatase and blood serum transaminase activity, decreased the number of sulfhydryl groups in whole blood, markedly decreased alkaline phosphatase, but moderately increased acid phosphatase in the liver and kidneys. The liver and

kidneys showed decreased concentrations DPN, TPN, succinic dehydrogenase, glucose-6-phosphatase and alpha-glycerophosphate. At 0.001 mg/kg, there were no measurable changes.

The results of the studies described above along with other experiments are summarized in Table V-2. Lifetime exposure studies have been conducted which were designed primarily to evaluate the carcinogenic potential of chlorobenzene. These studies will be discussed in the section on Carcinogenicity.

Teratogenicity

No teratogenicity studies were found in the peer-reviewed literature for chlorobenzene. However, Dow Chemical Company submitted a protocol for such a study in Fischer 344 rats and New Zealand white rabbits to its sponsor, the Chemical Manufacturers' Association and to EPA (Johns, et al., 1980) and has since completed and reported the studies to EPA.

Johns, et al. (1980) reported on the results of the preliminary study conducted to determine the doses to be used in the final study protocol. Groups of pregnant rats and rabbits were exposed via inhalation to 0, 300, 1000 or 3000 ppm CB 6 hours/day on days 6-15 of gestation (rats) or days 6-18 of gestation (rabbits). All ten of the rats exposed to 3000 ppm died or were sacrificed in moribund condition before the exposure period was over. Four of seven rabbits also died. The three survivors showed depressed body weight gain. One of the three rabbit litters contained only implantations undergoing resorption, suggesting embryotoxic effects.

At inhalation exposures of 1000 ppm, rats and rabbits

Table V-2

Chlorobenzene Toxicity Studies
(Subacute Exposure)

Species	Dose and Duration	Effect	Reference
Rats	1140 mg/kg/day for 5 days (oral)	Increased urinary coproporphyrin porphobilinogen, delta-aminolevulinic acid; fatty and necrotic liver damage.	Rimington & Zeigler, 1963
Rats	1 cc/day, 3 days (subcu. or i.v.)	Leukopenia	Cameron, et al., 1937
Rabbits	0.9 mg/kg/injection up to 20 injections (subcu.)	Swelling of convoluted tubule epithelium and glomeruli in kidneys, cellular swelling and fat deposition in liver.	Rozenbaum, 1947
Mice	2.5 mg/l, 7 hours/ day for 3 weeks (Inhalation)	Leukopenia, lymphocytosis, fatty degeneration in liver	Zub, 1979

Table V-2 (Continued)

Chlorobenzene Toxicity Studies
(Oral* Subchronic)

Species	Dose and Duration	Effect	Reference
Rats	0.1, 0.01+, 0.001 mg/kg/day for 9 mo.	Statistically significant inhibition of erythropoiesis, thrombocytosis and mitotic activity in marrow; inhibited higher cerebral cortex activity, neutropenia	Varshavaskaya, 1967
Dogs	27.25, 54.5+ or 272.5 mg/kg/day 5 days/week for 13 weeks	Diarrhea, vomiting, conjunctivitis. Cachexia; icterus; hepatic and renal discoloration; distended gall bladder; increased organ/body weight ratios for internal organs; death; increased numbers of monocytes; decreased blood sugar level and number of leukocytes. Immature leukocytes, low blood sugar elevated SGPT and alkaline phosphatase, increased total bilirubin and total cholesterol, gross and/or microscopic pathology of liver and kidney	Knapp, et al., 1971
Rats	12.5, 50+ or 250 mg/kg/day for 93 to 99 days	Retarded growth of male rats (high level). Liver and kidney weights significantly increased.	Knapp, et al., 1971

* Administered by gavage or capsule
\+ Lowest adverse effect dose reported.

Table V-2 (Continued)

Chlorobenzene Toxicity Studies
(Subchronic Inhalation)

Species	Dose and Duration	Effect	Reference
Rats	0.1 or 1.0 ug/L for 70-82 days, continuously	Encephalopathy, inflammation of internal organs, protein dystrophy in liver, regenerated cells in liver, focal giant-cell hyperplasia in kidneys.	Khanin, 1969
Rats	1.0 ug/L, for 60 days, continuously	Distorted chronaxie of antagonist muscles, increased blood cholinesterase activity, lowered alpha-globulin content of blood serum.	Tarkhova, 1965
Rats	0.1 mg/L, 7-14 weeks	Chronaximetric inhibition preceding enzymatic changes.	Pislaru, 1960
Rats	0.1 mg/L for 3 hrs., alternate days for 37 weeks.	Temporary inhibition of extensor tibialis chronaxie after 7-14 weeks	Gabor and Raucher, 1960

Table V-2 (Continued)

Chlorobenzene Toxicity Studies
(Subchronic Inhalation)

Species	Dose and Duration	Effect	Reference
Dogs	0, 0.75, 1.50 or 2.00+ mg/L, 6 hr/day, 5 days/week, 13 weeks	Enlarged and hardened liver, vacuolated hepatocytes, cytoplasmic vacuolation of renal collecting tubules, bilateral atrophy of seminiferous tubules, lower total leukocyte counts, elevated serum AP, GOT, GPT.	Industrial Biotest for Monsanto, (IBT,1979)
Dogs	0, 0.78, 1.57+ or 2.08 mg/L, 6 hr/day, 5 days/week for 6 months	Increase in emesis and abnormal stools; intermittent increases in blood alkaline phosphatase; statistically-significant decrease in adrenal weights and increase in liver/body weight ratio at two highest doses.	Monsanto, 1980
Rats and Rabbits	0, 75 or 250 ppm (0.075 or 0.25 mg/L) 7 hr/day, 5 days/week, up to 120 exposure days	Trend toward microcytic anemia, decrease in SGOT activity, focal lesions of adrenal cortex, tubular lesions in kidney, congestion in liver and kidneys in rats.	Dilley, 1977
Mice	0.1 mg/L, 7 hrs/day, 3 months	Leukopenia, lymphocytosis	Zub, 1979

+ Lowest adverse effect dose reported.

exhibited decreased food consumption and body weight gain. There was a statistically significant increase in liver:body and kidney:body weight ratios in the rats. Also, in rats, a statistically significant increase in resorption incidence occurred, again indicating embryolethality. Rabbits showed a slight, but not statistically significant, increase in resorptions. At 300 ppm, no evidence of significant toxicity in either dams or embryos was observed.

The full teratology study was conducted in two phases (Hayes, et al., 1982). In Phase I, bred rats and inseminated rabbits were exposed via inhalation to 0, 75, 210 or 590 ppm CB, 6 hours/day during the period of major organogenesis (days 6-15 for rats; days 6-18 for rabbits). Rat groups contained 32 or 33 animals; rabbits groups numbered 30 animals.

Animals were observed daily for overt toxicity and weighed on days 6,9,12,16 and 21 (rats) or days 6,9,12,15,19 and 29 (rabbits). Food and water consumption of rats were recorded every third day beginning on Day 6 of gestation. Maternal liver weights in both species were determined at sacrifice (day 21 for rats; day 29 for rabbits). The following data also were recorded: 1) number and position of fetuses in utero; 2) number of dead and live fetuses; 3) number and position of resorption sites; 4) number of corpora lutea; 5) sex, body weight and crown-rump length of each fetus, and 6) any gross external alterations.

Among the rats, no maternal deaths or overt toxicity occurred in animals of any dose group during the exposure period. Nevertheless, some evidence of maternal toxicity was

exhibited decreased food consumption and body weight gain. There was a statistically significant increase in liver:body and kidney:body weight ratios in the rats. Also, in rats, a statistically significant increase in resorption incidence occurred, again indicating embryolethality. Rabbits showed a slight, but not statistically significant, increase in resorptions. At 300 ppm, no evidence of significant toxicity in either dams or embryos was observed.

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Animals were observed daily for overt toxicity and weighed on days 6,9,12,16 and 21 (rats) or days 6,9,12,15,19 and 29 (rabbits). Food and water consumption of rats were recorded every third day beginning on Day 6 of gestation. Maternal liver weights in both species were determined at sacrifice (day 21 for rats; day 29 for rabbits). The following data also were recorded: 1) number and position of fetuses in utero; 2) number of dead and live fetuses; 3) number and position of resorption sites; 4) number of corpora lutea; 5) sex, body weight and crown-rump length of each fetus, and 6) any gross external alterations.

Among the rats, no maternal deaths or overt toxicity occurred in animals of any dose group during the exposure period. Nevertheless, some evidence of maternal toxicity was

seen in the high dose group (i.e.,decreased body weight gain and increased absolute and relative liver weights). Food consumption also was decreased significantly in this group on days 6-8, but was increased significantly on days 15-17. This phenomenon was not considered to be toxicologically significant. Water consumption in the high dose group was elevated significantly on days 9-17.

No effects were seen in rats on pregnancy rates, mean litter size, incidence of implantations undergoing resorption, fetal sex ratios or fetal body measurements at any dose level. The incidence of major malformations, whether considered collectively or singly, was not increased significantly for any treatment groups when compared with the control group. There were statistically significant decreases in the incidence of focal necrosis in the liver of fetuses from the two higher dose groups and in the incidence of slight cerebral hemorrhage in the low dose group. The investigators did not consider these changes to be toxicologically significant.

There was an increased incidence in certain minor skeletal variants in the rat fetuses. The incidence of delayed ossification of the centra of the cervical vertebrae was increased significantly in the low and high dose groups. Also, there was a statistically significant increase in the occurrence of bilobed centra of the thoracic vertebrae and a decrease in the incidence of spurs in the fifth cervical vertebrae in the high dose group. The investigators concluded that these findings were not toxicologically significant or indicative of a teratogenic response.

Two maternal deaths occurred in rabbits during the Phase I study. One control female died on day 18 and one from the low dose group on day 29. Four females delivered early (2 controls, 1 exposed at 75 ppm and 1 at 210 ppm). Another animal exposed to 210 ppm died of pneumonia during delivery.

There were signs of maternal toxicity in pregnant rabbits exposed to 210 or 590 ppm. Statistically significant increases in absolute and relative liver weights were observed. Significant differences in these parameters were not observed in the low dose group when compared with the controls. No significant differences were noted with respect to body weight or body weight gain.

Pregnancy rates in the rabbits were not affected in treated animals, nor were mean litter size or incidence of implantations undergoing resorption. Statistically significant increases in the percent preimplantation loss was observed in the low and intermediate dose groups, but since exposure was not initiated until day 6 of gestation, this phenomenon was considered to be unrelated to exposure to monochlorobenzene.

A statistically significant difference in fetal sex ratio (58% male:42% female) was observed in the litters from the low dose group. A significant increase in the mean fetal crown-rump length in litters from the intermediate dose group also was seen. The changes were not considered toxicologically significant. Mean fetal body weights/litter were not altered significantly.

The incidences of external, soft tissue or skeletal alterations among litters from exposed rabbits did not differ

significantly from controls. Whether considered singly or collectively, the incidences of major malformations observed in each of the exposed groups were not significantly different from the controls. However, the variety and number of major malformations in the control group occurred at levels slightly higher than the laboratory's historical controls. Thus, it was decided that the rabbit teratology study would be repeated. The study was repeated as Phase II.

The protocol used in Phase II was essentially the same as that used in Phase I, except that the dose levels employed were 0, 10, 30, 75 and 590 ppm.

No maternal deaths occurred during the Phase II study. The only sign of maternal toxicity was a statistically significant increase in absolute liver weights in the high dose group. The liver:body weight ratio was not altered significantly at this or any other exposure level. No differences in maternal body weights or body weight gain occurred.

Again, statistically significant increases in percent pre-implantation loss were observed, both in the 75 and 590 ppm dose groups. Also, a significant increase in the percentage of implantations undergoing resorptions occurred in the 590 ppm group when compared with the controls (61% of the litter representing 12% of the fetuses in the 590 ppm group vs. 41% of the litters representing 6% of the fetuses in the control group). The investigators did not consider this observation to be toxicologically significant, stating that the 61% was within the normal range for that laboratory. Fetal sex ratios and body measurements were not altered significantly.

The incidences of major malformations in the exposed groups, whether considered collectively or singly, were not significantly different from the incidences observed in the controls. In fact, statistically significant decreases were seen in the 10 and 590 ppm groups.

The investigators concluded that, under the conditions of the studies, inhalation of CB for 6 hours/day at exposures up to 590 ppm during the period of major organogenesis was not teratogenic to rats and rabbits.

Mutagenicity

Abnormal mitotic division of the onion, Allium cepa, on treatment with several halogenated benzenes has been described by Ostergran and Levan (1943). Chlorobenzene at concentrations of 1000×10^{-6} mol caused full c-mitosis disturbances.

The spores of the vitamin B_1-deficient mutant Streptococcus antibioticus 400 were treated with chlorobenzene (0.05 and 0.1 ml). After 24 hours, all spores were dead. At six hours, the maximum yield of back mutations observed exceeded the controls by a factor of 187 (low dose) and 1400 (high dose). The mutagenic activity was estimated from the frequency of induced reverse mutations from auxotrophic to prototrophic phenotype (Koshkinova, 1968).

Prasad and Pramer (1968) and Prasad (1970) investigated the mutagenic potential of chlorobenzene at a concentration of 200 ug/ml. The chemical was evaluated by determining the frequency of back mutation of the methionine-requiring locus in the fungus, Aspergillus nidulans. No metabolic activation was

employed. Chlorobenzene was nonmutagenic under these expermental conditions.

Chlorobenzene was nonmutagenic when tested for gene mutation in the Salmonella/microsomal assay (Monsanto 1976a; Dupont, 1977; Merck, 1978 and Simmon, et al., 1979). The compound was tested in Salmonella strains TA 1535, 1537, 1538, 98 and 100 and E. coli WP2 with and without microsomal activation, at concentrations in the medium of 0.01 to 5.0 ul/plate or 150-3000 ug/plate. Significant cytotoxicity was observed at many of the dose levels in most of the strains. For this reason, it would be difficult to accept the study results as proof of negative mutagenic properties since the level of cytotoxicity may have masked the expression of revertants. Statistical analyses of the data in these studies did not include numeration of the number of revertants per unit of survivors, an analysis which might provide a better indication of the effect of the compound on the test organisms.

The potential of chlorobenzene to induce specific locus forward mutations was tested in the mouse lymphoma L5178Y cell culture assay system (Monsanto, 1976b). The compound was nonmutagenic at 0.001 ul/ml without enzymatic activation and at 0.001-0.01 ul/ml with activation.

Chlorobenzene was shown to induce reciprocal recombination in the yeast Saccharomyces cerevisiae strain D3 (Simmon, et al., 1979). In the presence of the microsomal activation system, at concentrations of 0.05 and 0.06%, chlorobenzene caused a reproducible increase in the number of recombinants/10^5 survivors when compared with controls.

At concentrations of 10 or 20 ul/plate, chlorobenzene was equally toxic to the repair-proficient (pol A^+) and repair-deficient (pol A^-) strains of E. coli and to rec^+ and rec^- strains of Bacillus subtilis (Simmon, et al., 1979).

Carcinogenicity

Chlorobenzene has undergone study in the NTP Bioassay Program to determine its carcinogenic potential. All studies have been completed. A draft report of the 104-week chronic study is now available. All studies were conducted by Battelle's Columbus Laboratories with Fischer 344 rats and B6C3F1 mice (Battelle's Columbus Laboratories, 1977 a, b, 1978 a, b, c, d).

In the acute toxicity study, rats and mice (five animals of each sex/species/dose level) were gavaged with single doses of 0, 250, 500, 1000, 2000 or 4000 mg CB/kg in corn oil and observed for up to 14 days (Battelle, 1977 a, b). At the end of the 2-week observation period, survivors were sacrificed without necropsy or histopathological examination. No clinical chemistry studies were done. On the second day, 3 of 5 male and 4 of 5 female rats receiving 4000 mg/kg died. One male rat in the 2000 and 500 mg/kg groups died on day 2. Two females in the 1000 mg/kg group died on day 3. Of the mice, 19 males and 14 females died during the course of the study. All males from the three highest dose groups died on day 2 or 3. One in the 500 mg/kg group died by day 2 and three from the lowest dose group died on day 2 or 3. All female mice in the two highest dose groups were dead by day 6. Two of the 1000 mg/kg-dosed group were dead by day 3 and two

others at 250 mg/kg died later. Thus, it would seem that mice were more severely affected by their acute exposure to CB than were rats, with male mice being more susceptible than the females.

Based upon the results of the acute toxicity study, doses were selected for repeated oral dose studies. Mice were gavaged once daily for 15 days with doses of 0, 30, 60, 125, 250 or 500 mg/kg CB in corn oil (5 animals/sex/dose level) (Battelle, 1978a). Five males and one female died during the course of the study, but none of the deaths were considered to be treatment related. No treatment-related effects were noted in the survivors. Rats were gavaged once daily for 15 days with doses of 0, 125, 250, 500, 1000 or 2000 mg/kg CB in corn oil (Battelle, 1978b). A total of 10 males and females died during the course of the 15-day study. All five males and all five females at the highest dose level died by day 4 or 6, respectively. All five animals of each sex at 1000 mg/kg died by day 15. The only significant finding upon gross inspection was that of pale livers (yellow atrophy), indicating hepatotoxicity. This finding was observed in those rats at the two highest dose levels. The 500 mg/kg dose was judged to be the no-effect level in both species.

Subchronic toxicity studies were conducted in which rats and mice were gavaged five times weekly for 13 weeks with 0, 60, 125, 250, 500 or 750 mg/kg MCB in corn oil (10 animals/species/sex/dose level) (Battelle, 1978 c,d). Animals surviving the 91 days of treatment were subjected to full necropsies, weighing of organs and histopathological evaluation. Additional studies performed at the end of the exposure period included urinalysis

(only of animals at the highest surviving dose and of controls), hematology and clinical chemistry.

Among the 60 mice, 26 males and 22 females died prior to the scheduled sacrifice. All ten males receiving 500 or 750 mg/kg died within the first week of the study. Six males in the 250 mg/kg dose group died by week 10. All 10 females at 750 mg/kg died by week 9; seven females in the 500 mg/kg group and four in the 250 mg/kg group died during the last three weeks. The rats had a higher survival rate. Fourteen males and 11 females died before the study ended. No males at 750 mg/kg died early,but four died at the 500 mg/kg level. One control died in week 9. The first male died in week 1, the first female succumbed in week 6.

While average individual weekly food consumption by the mice did not differ among the experimental groups, body weight gain was significantly reduced over the entire course of exposure in certain of the treated groups when compared with controls. In males, the percentage difference in body weight gain was -74% in the 250 mg/kg group and -25% and -27%, respectively, in the 60 and 125 mg/kg groups. Among the females, there was a -63% difference at 500 mg/kg, -51% at 250 mg/kg, but no statistically-significant difference at 60 or 125 mg/kg.

Among the 60 rats, 25 died early (14 males and 11 females). Deaths included 9 of the 10 males at the 750 mg/kg dose, 4 of the 10 at 500 mg/kg and 1 control. Eight of 10 females receiving 750 mg/kg died along with 3 of 10 at 500 mg/kg.

In contrast to the mice, both male and female rats at the two highest doses showed a decrease in food consumption. Males

at 750 mg/kg ate 21% less than controls, males at 500 mg/kg, 9% less. Females at 750 mg/kg ate 2.2% less; females at 500 mg/kg ate 13% less. Differences in body weight gain increased as the treatment dose increased, with a 10% difference at 250 mg/kg and 22% at 750 mg/kg (representing the sole survivor) for males compared with the controls. In females, 25% and 51% differences were seen at 500 and 750 mg/kg, respectively.

No clinically-significant chlorobenzene-related changes were observed in any of the hematological parameters measured in either species. None of the clinical chemistry parameters measured were altered in the mice. However, in the rats, SGPT and gamma-glutamyl transpeptidase (GGPT) levels were elevated in the two females and one male surviving after treatment with 750 mg/kg. Urinalyses of the controls and survivors from the 500 and 740 mg/kg groups revealed a dose dependent polyuria with concomitant decreases in specific gravity and creatinine concentration. Also, at the two highest doses, urinary coproporphyrin excretion was increased in both sexes of rats. In mice, the increase was observed only in females at 250 and 500 mg/kg.

Liver:body weight ratios were significantly increased in mice of both sexes at 500 mg/kg and higher. Some increase (not statistically significant) was seen in males at 125 mg/kg. Both male and female rats at 250 and 500 mg/kg showed a significant increase. Mice and rats at the three highest doses all exhibited significant histopathological changes consisting of hepatic necrosis, nephrosis, myeloid depletion, lymphoid depletion and lymphoid necrosis. Minimal histopathological changes were seen

in male mice at 125 mg/kg.

Doses for the chronic study were selected on the basis of the findings observed in the subchronic studies. Both sexes of rats and female mice received 60 or 120 mg/kg/day; male mice received 30 or 60 mg/kg/day. Doses were administered by gavage in corn oil, 5 days/week for 103 weeks. Groups of 100 rats and 100 mice (50 of each sex) served as vehicle controls, receiving corn oil only on the same schedule as the treated animals. Other groups of 100 rats and 100 mice served as untreated controls.

The following information is presented in the draft NTP technical report on the bioassay for chlorobenzene (NTP, 1983).

Mean body weights of dosed animals were essentially the same or greater than those of the controls throughout the study. Survival of low-dose male rats, all female rats and mice and high-dose male mice was not affected adversely by exposure to chlorobenzene (see Tables V-3 and V-4). The survival rate for the high-dose male rats (26/50, 52%) was significantly lower ($P<0.014$) than for the vehicle controls (39/50, 78%). The survival rate for low dose male mice was signficantly lower than vehicle controls (28/50 or 56% compared with 39/50 or 78%, respectively).

According to the original histopathological evaluation, there was an apparent increase in the occurrence of hepatocellular necrosis and decreases in the occurrence of hepatocellular basophilic change and granulomatous inflammation in treated rats of both sexes. Review of the slides by a second pathologist determined that there was no significant difference. Both individuals

Table V-3

Rat Survival Rates at 104 Weeks

(NTP, 1983)

Exposure Dose (mg/kg/day)	Males		Females	
	Number	Percentage	Number	Percentage
0 (Untreated)	34/50*	68%	37/50	74%
0 (Vehicle)	39/50	78%	29/50	58%
60	32/50	64%	30/50	60%
120	26/50	52%†	31/50	62%

†P<0.014, when compared with vehicle controls

Table V-4

Mouse Survival Rates at 105 Weeks

(NTP, 1983)

Exposure Dose (mg/kg/day)	Males		Females	
	Number	Percentage	Number	Percentage
0 (Untreated)	35/50*	70%	37/50	74%
0 (Vehicle)	39/50	78%	40/50	80%
30	28/50	56%°		
60	29/50	58%	41/50	82%
120			38/50	76%

°P<0.031, when compared with vehicle controls

* $\frac{\text{Number of survivors}}{\text{Number of animals at start of study}}$

agreed that the necrotic lesions were minimal to mild in severity in both chlorobenzene-treated groups. Therefore, it was concluded that the evidence for mild chlorobenzene-induced hepatocellular necrosis in this study is considered equivocal.

Neoplastic nodules occurred in male rats with a significant positive trend. The incidence of animals with nodules was significantly higher in the high-dose males than in the vehicle control males, by all three tests: life table analysis, incidental tumor test and Cochran-Armitage trend and Fischer exact tests. An increased incidence of hepatocellular carcinomas were not observed in the treated males; however, the combined incidences of nodules and carcinomas were increased by life table analysis in the high-dose group. No significant increases in carcinomas or nodules, singly or combined, were seen in females.

Interstitial tumors of the testis were observed with a positive trend in the life table test, and the incidence in the high-dose males was significantly higher than in the vehicle controls by the life table test, but not by either the incidental tumor or Fisher exact tests. Adenomas in the female rats, and adenomas, adenocarcinomas or carcinomas (combined) in male rats occurred with a significant negative trend. The incidences in the high-dose groups were significantly lower than those in the controls. Endometrial polyps of the uterus were observed with a significantly lower incidence in the low-dose group than in the control females.

Among the mice, the survival of the low-dose males was significantly lower than that of the controls ($P<0.031$).

No site-specific tumors or non-neoplastic pathology occurred at statistically-significant increased or decreased incidences in treated mice of either sex.

The NTP has concluded tentatively that the doses used in these chronic studies were adequate for carcinogenicity. In addition, NTP proposed that under the conditions of the test, chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in the high-dose male rats, providing some but not clear evidence of carcinogenic activity in male rats. Carcinogenic effects of chlorobenzene were not observed in female rats or mice of either sex.

Multiple Chemical Exposures: Actions of Combinations

The halogenated benzenes, as a group, appear to interact with cytochrome P-450 in animal species which have been investigated. These chemicals appear to stimulate and inhibit drug metabolism and to alter the levels of cytochromes in cells. Since many compounds are metabolized by the cytochrome P-450 system, there are many possibilities for additive, synergistic or antagonistic actions when CB is encountered in combination with other chemicals.

There was one example of synergistic effects of halogenated benzenes on a target organism found in the literature (Hinze, et al., 1970). The antifungal activity of halogenated benzenes was synergistic with organo-tin compounds. The mechanism of the toxicity was not discussed. There were no studies found on synergistic effects in mammals or other nonmammalian species.

One example of an additive effect with concomitant exposure was reported by Shelton and Weber (1980) in the mouse. A variety of mixtures of carbon tetrachloride and chlorobenzene or carbon tetrachloride and acetaminophen was administered to male albino CD-1 mice. A positive quantal response was considered to be an elevation of plasma alanine aminotransferase activity at least three standard deviations above control. Dose response curves were developed for each compound separately and the slopes compared with one another. From these data, theoretical dose response curves for the mixtures were postulated with the prediction that the interaction would be additive in both cases. In the case of the combination of carbon tetrachloride and chlorobenzene, the observed dose response curve did not deviate from the curve predicted on the basis of an additive effect ($P>0.975$). Therefore, the authors classified the carbon tetrachloride:chlorobenzene interaction as concentration additive.

VI. HEALTH EFFECTS IN HUMANS

Most of the incidents reported in the literature are poisonings resulting from inhalation of chlorinated benzenes, including chlorobenzene. Accidental inhalation can occur either at home or at work. There are several cases reported, however, in which CB was accidentally or deliberately ingested. (See Table VI-1).

Inhalation exposure to CB causes headaches, irritation of the eyes and upper respiratory tract, sedation and narcosis (Girard, et al., 1969; Smirnova and Granik, 1970). A two-year-old male survived ingestion of 5-10 cc CB. When hospitalized, he was unconscious, cyanotic and twitching in the head and neck regions (Reich, 1934). No long-term follow-up of this patient was made.

Rozenbaum, et al. (1947) studied 28 workers exposed to variable concentrations of CB vapors. A number of the workers exhibited symptoms that included headache, dizziness, somnolence and dyspeptic disorders. Spastic contractions of finger muscles were observed in nine people; hyperthesia of the hands was found in four people. Two hematological examinations of the workers were conducted. The first revealed normal red cell conditions, but the number of white cells were abnormally low in 11 people. Seven people exhibited thrombocytopenia with two of these individuals suffering from bleeding of the gums and nasal mucosa. Several months later the tests were repeated. The investigators did not state if the workers continued to be exposed during this period. The abnormalities seen in the first examination had returned to nearly normal.

Gabor, et al.(1962) reported on individuals who were exposed to benzene, CB and vinyl chloride during a three-year observation period. Eighty-two workers examined at 1-year time points were found to have an increase in the activity of leukocyte peroxidase at all three sampling times and a slight leukopenia toward the end of the third year.

Ehrlicher (1968) reported that massive hemoptysis occurred in a male worker exposed to a large amount of chlorobenzene sprayed into the atmosphere during an industrial accident. Onset of the bleeding did not occur until several hours after inhalation of the compound. The hemorrhaging was preceded by a feeling of general discomfort. No follow-up studies on this patient were reported.

Girard, et al. (1969) reported the case of the elderly female exposed to a glue containing 70% CB for a period of six years. She presented with symptoms of headache, irritation of the eyes and upper respiratory tract and was diagnosed to have medullary aplasia. Smirnova and Granik (1970) reported on three adults who developed numbness, loss of consciousness, hyperemia of the conjunctiva and the pharynx following exposure to "high" levels of CB. Information concerning the ultimate course of these individuals is not available.

Dunaeveskii (1972) reported on the occupational exposure of workers exposed to the chemicals involved in the manufacture of chlorobenzene at levels below the allowable levels (not stated). After over three years' exposure, cardiovascular effects were noted as pain in the area of the heart, bradycardia, irregular

variations in the electrocardiogram, decreased contractile function of myocardium and disorders in adaptation to physical loading. Filatova et al. (1973) reported on the prolonged exposure of individuals involved in the production of diisocyanates to the factory air which contained MCB as well as other chemicals. Symptoms noted included asthmatic bronchitis, sinus arrythmia, tachycardia, arterial dystrophy and anemic tendencies.

Special Groups at Risk

Petrova (1972) studied the course of pregnancy and deliveries in women exposed to air in a varnish manufacturing factory where the air contained three times the maximum permissible level of CB but also which included toluene, ethyl chloride, butanol, ethyl bromide and orthosilisic acid ester. The only reported significant adverse effect of this mixed exposure was toxemia of pregnancy.

Table VI-1

Human Exposure To Chlorobenzene

(Modified from Ware and West, 1977)

Subject	Exposure	Symptoms	Clinical Report	Follow-up Studies	Reference
70 year old female	1958-1964 to a glue containing 70% CB (no benzene or higher homologues)	Headaches, irritation of eyes and upper respiratory tract	Medullar aplasia	1966-"satisfactory" 1977-"mediocre"	Girard, et al., 1969
2 year old male	Swallowed CB, 5-10 cc	Unconscious, no reaction to skin stimuli, muscle spasms, cyanotic	CB intoxication	Odor of CB present in urine and air exhaled 5-6 days later. General health appeared undisturbed	Reich, 1934
3 adults	Occupational exposure to CB	Numbness, inhibition, loss of consciousness, hyperemia of conjunctivae, sclera, pharynx	CB intoxication	Follow-up studies were performed, data not clear on results	Smirnova and Granik, 1970
28 workers	Occupational exposure to CB	headache, dizziness, somnulence, dyspeptic disorders	CB intoxication	Abnormal white cell counts in 11 which reverted to normal after termination of exposure	Rozenbaum, et al., 1947
Male worker	Inhalation of CB	General discomfort, massive hemoptysis several hours later	Poisoning due to CB	Not indicated	Ehrlicher, 1968

VII. MECHANISMS OF TOXICITY

Many workers have studied the possibility that organ and cellular damage caused by many compounds is mediated via chemically reactive metabolites. Many of the metabolites formed are chemically inert, but certain of the metabolites such as the arene oxides or epoxides may interact with physiological or biochemical processes, causing either pharmacological or toxicological effects.

There is strong evidence that the metabolism of chlorobenzene and other halogenated benzenes proceeds through the formation of arene oxide intermediates. Such evidence includes: 1) a correlation of overall toxicity and cellular toxicity with metabolism; 2) a correlation of toxicity with *in vivo* and *in vitro* covalent binding of metabolites with proteins and 3) the appearance of toxicity associated with a depletion of tissue levels of glutathione.

While the arene oxides of chlorobenzene have not been isolated *in vivo*, evidence suggests that the oxides are formed during the metabolism of the parent compound to 2- and 4-chlorophenol which have been isolated as metabolites (Selander, *et al.*, 1975b). These investigators synthesized the 3- and 4-chlorobenzene oxides. Isomerization of each oxide was examined in the presence of rat liver microsomes at pH 8. Only 4- and 2-chlorophenols (>99.5%) were produced from each oxide, respectively. While it has been shown that 3-chlorophenol also is produced *in vivo* (Smith, *et al.*, 1972) and by the perfused liver (Selander, *et al.*, 1975a), this isomer appears to result from the action

of an enzyme which catalyzes the insertion of an oxygen directly into the carbon-hydrogen bond without intervention of an arene bond (Tomaszewski, et al., 1975).

Selander, et al. (1975b) performed trapping experiments in which ^{14}C-chlorobenzene was incubated with liver microsomes from control rats or those pretreated with 3-methylcholanthrene (3-MC) or phenobarbital (PB) in the presence of the 4- or 3-chlorobenzene oxide. Recovery of the two oxides was 47% and 29%, respectively.

Studies of other halogenated benzenes (HBs) (Brodie, et al., 1971; Reid, et al., 1971; Mitchell, et al., 1971) also demonstrated that hepatic necrosis produced on exposure to these compounds results from their conversion to reactive toxic intermediates. The stimulation of metabolism by pretreatment with phenobarbital potentiates hepatic damage in rats, as can be seen in Table VII-1. Conversely, blocking metabolism by SKF-525A (2-diethylaminoethyl-2, 2-diphenylvalerate hydrochloride) or piperonyl butoxide (a pesticide synergist) prevents HB hepatotoxicity (Reid, et al., 1973; Reid and Krishna, 1973).

The severity of liver necrosis correlates closely with the extent of covalent binding of metabolites in vitro and in vivo after various pretreatments, as can be seen in Table VII-2. These studies also have demonstrated that the arene oxide formed is conjugated with glutathione (Reid, et al., 1973; Reid and Krishna, 1973). The glutathione content in the liver is decreased in the presence of large doses of the more active halobenzenes (see Table VII-2). Conjugation with glutathione is believed to be an important

Table VII-1

Effect of Phenobarbital and SKF-525A Administration on Covalent Binding of Halogenated Benzene Derivatives to Rat Liver Protein in Vivo 6 Hours after Administration (Reid and Krishna, 1973)

	Control (nmols/mg protein ±S.E.)	Phenobarbital 80 mg/kg) (nmols/mg protein ±S.E.)	Phenobarbital SKF-525A* (nmols/mg protein ±S.E.)
Monobromobenzene-^{14}C (1 mmol/kg)	0.267±0.034	0.550±0.031[a]	0.360±0.024[b]
Monochlorobenzene-^{14}C (1 mmolM/kg)	0.364±0.053	1.268±0.278[a]	0.483±0.144[c]
Monoiodobenzene-^{14}C (1 mmolM/kg)	0.090±0.015	0.545±0.129[a]	0.666±0.304
Monofluorobenzene-^{14}C (1 mmolM/kg)	0.085±0.015	0.054±0.008	0.052±0.005
o-Dichlorobenzene-^{14}C (0.5 mmol/kg)	0.234±0.015	0.308±0.038	0.186±0.014[c]
p-Dichlorobenzene-^{14}C (0.5 mmolM/kg)	0.021±0.002	0.012±0.001[a]	0.006±0.001[b]

* 2-Diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF-525A) (75 mg/kg i.p.) was given 2 hours before the hepatotoxin.

Values are the mean of 6 rats ±S.E.

[a] $p<0.01$ compared with controls
[b] $p<0.01$ compared with phenobarbital alone.
[c] $p<0.02$ compared with phenobarbital alone.

Table VII-2

Covalent Binding, Hepatotoxicity, and Mercapturic Acid Excretion of Halogenated Benzene Derivatives
(Reid, et al., 1971; Reid, et al., 1973; Reid and Krishna, 1973)

Compound		Covalent binding (nmols/mg protein ±S.E.)	Hepatic necrosis	Mercapturic acid excretion	Glutathione concentration (% of control)*
Monobromobenzene	(1 mmol/kg)	0.534±0.050[a]	Yes	3+	67
Monochlorobenzene	(1 mmol/kg)	0.604±0.044[a]	Yes	3+	Not determined
Monoiodobenzene	(1 mmol/kg)	0.323±0.054	Yes	3+	66[b]
Monofluorobenzene	(1 mmol/kg)	0.060±0.004[a]	No	+	82
o-Dichlorobenzene	(0.5 mmol/kg)	0.234±0.015[c]	Yes	2+	48[b]
p-Dichlorobenzene	(0.5 mmol/kg)	0.021±0.002[c]	No	±	101

Values are the mean ± S.E. of 6 rats.

* Glutathione concentration determined 3 hours after administration of the hydrocarbon.

a Sacrificed at 24 hours.

b $p<0.01$ compared with control.

c Sacrificed at 6 hours.

detoxication mechanism. Liver necrosis occurs when glutathione is depleted.

More recently, Burka, et al. (1983) studied the monohydroxylation of halobenzenes, including chlorobenzene, by phenobarbital-induced rat liver microsomes. In the case of chlorobenzene, p-chlorophenol was found to be the major metabolite, as has been observed *in vivo*. The authors found a good correlation of V_{max} with the σ^+ Hammett constant, implying that a positively-charged transition state exists. This suggests that the product-determining step occurs after the rate-determining step. The rat-determining step is postulated to be the formation of a radical cation intermediate.

Just as there are species differences in metabolism, there are differences in the rate and/or extent of metabolism of a halogenated benzene in different organs, i.e., lung may differ from kidney which differs from liver, etc. (see Table VII-3). As can be seen, phenobarbital does not increase covalent binding in the lung as shown for the liver (Reid, *et al.*, 1973; Reid and Krishna, 1973).

Phenobarbital or 3-methylcholanthrene enhances the activity of Δ-aminolevulinic acid (Δ-ALA) synthetase, the rate-limiting factor in the enzymatic synthesis of porphyrins. Some porphyrins are intermediates in the synthesis of cytochromes. Porphyria, a disturbance in porphyrin metabolism, is characterized by increased formation and excretion of porphyrin precursors, cutaneous photosensitivity, frequent hemolytic anemia and splenomegaly. All of the chlorinated benzenes have been shown to pro-

Table VII-3

Binding of Aromatic Hydrocarbons in Rat Lung:
Effect of Phenobarbital*
(Reid, et al., 1973)

Compound	Time Killing (hour)	Binding of Hydrocarbon in Lung (nmol/mg protein) Control	Phenobarbital
Bromobenzene-^{14}C, 1mM/kg	6	141 ± 18	77 ± 7.6[a]
	24	117 ± 14	88 ± 7.4
Chlorobenzene-^{14}C, 1mM/kg	6	268 ± 60	172 ± 20
	24	164 ± 26	91 ± 4.0[b]
o-Dichlorobenzene-^{14}C, 0.5mM/kg	6	27.3 ± 1.4	18.9 ± 2.7[c]
	24	20.9 ± 2.4	15.3 ± 2.2
p-Dichlorobenzene-^{14}C, 0.5mM/kg	6	4.6 ± 0.2	3.4 ± 0.5
	24	3.4 ± 0.4	2.8 ± 0.2

* Values are the means ±S.E. of 6 animals

a $p<0.01$

b $p<0.02$

c $p<0.05$

duce disturbances in porphyrin metabolism.

Rimington and Ziegler (1963) showed that, in rats, long-term oral administration of 500 to 800 mg/kg body weight of chlorinated benzenes (except pentachlorobenzene) lead to a hepatic porphyria characterized by elevated levels of precursor porphyrins in liver and feces. The first signs of intoxication included an increase in urinary coproporphyrin and porphobilinogen (PBG). Increased aminolevulinic acid excretion was a late effect. The most active compounds were p-dichlorobenzene, 1,2,4-trichlorobenzene, 1,2,3,4-tetrachlorobenzene (see Tables VII-4 and VII-5). Histologic examination of the animals' livers showed necrosis and fatty changes over large areas. Mono-, 1,2-di and 1,2,4-trichlorobenzene were the most toxic.

Ariyoshi, et al. (1975a,b) have shown an initial increase in content of cytochrome P-450 and an initial decrease in delta-ALA synthetase activity following administration of chlorinated benzenes. In the time course following a single oral dose of 250 mg/kg body weight, the activity of Δ-ALA synthetase decreased at six hours, was normal at 12 hours and increased at 24 hours. The opposite changes were noted in the content of cytochrome P-450 (see Tables VII-6 and VII-7).

In a later publication, the same group of investigators reported a dose-response relationship for the same parameters in a multiple dose study (Ariyoshi, et al., 1981). Animals were injected intraperitoneally with single doses of 50, 100 or 200 mg CB/ml/kg 24 hours before sacrifice. At the lowest dose, no significant increase in Δ-aminolevulinic synthetase activity

Table VII-4

Mean Peak Values of Urinary Porphyrins and Porphyrin Precursors Following Treatment of Male Rats with the Maximum Doses Tried of Each Chlorinated Benzene (Rimington and Ziegler, 1963)

No. of rats	Compound	Sol-vent	Max. dose (mg/kg)	Days on max. dose	Copro-porphyrin (ug/day)	Uropro-phyrin (ug/day)	PBG (ug/day)	ALA (ug/day)	ALA:PBG
	Controls*	C			4.3-6.8	0.1-0.3	2.5-6.5	38.7-51.6	
3	Monochlorobenzene	P	1,140	5	30.50	1.40	26.70	56.40	2.98
3	1,2-Dichlorobenzene	P	455	15	43.10	2.01	26.65	11.32	2.11
3	1,4-Dichlorobenzene	P	770	5	61.80	10.99	1328.10	437.41	0.36
3	1,2,3-Trichlorobenzene	C	785	7	36.59	0.72	57.38	36.26	1.01
3	1,2,4-Trichlorobenzene	C	730	15	58.31	2.73	179.00	145.70	1.06
3	1,2,3,4-Tetrachlorobenzene	P	660	10	28.96	3.80	520.63	315.78	0.61
6	1,2,4,5-Tetrachlorobenzene	C	905	5	4.10	0.22	6.50	18.08	6.69

P = liquid paraffin; C = 1% cellofas;

PBG = porphobilinogen; ALA = delta-aminolevulinic acid.

* Mean min. and max. of 5 rats during 41 days.

Table VII-5

Porphyrins, Porphobilinogen and Catalase Activity in Livers of Rats Treated with Chlorinated Benzenes (Rimington and Ziegler, 1963)

No. of rats	Compound	Max. dose	Days on max. dose	Copro-porphyrin (ug/100g)	Protopor-phyrin (ug/100g)	Uropor-phyrin (ug/100g uncorrected)	PBG	Catalase (m.eq.mg) net wt.
	Controls*			4.5	9.7	1.3	-	0.85
2	Monochlorobenzene	1400	5	Trace	13.00	6.35	-	0.502
2	1,2-Dichlorobenzene	450	15	10.05	34.80	14.40	-	0.364
2	2,4-Dichlorobenzene	770	5	5.07	60.35	60.35	-	0.880
2	1,2,4-Trichlorobenzene	780	7	2.65	3.55	20.00	-	0.857
5	1,2,4-Trichlorobenzene	500	10	42.56	55.60	52.70	++	0.747
3	1,2,3,4-Tetrachlorobenzene	660	10	35.04	56.57	41.32	+	0.772
2	1,2,3,5-Tetrachlorobenzene	850	5	6.30	9.90	2.15	-	0.838

PBG = porphobilinogen.

* Mean of 2 rats.

Table VII-6

Effect of Chlorinated Benzenes on -aminolevulinic Acid Synthetase and Cytochrome P-450 Content of Rat Liver
(Rimington and Ziegler, 1963; Poland, et al., 1971; Ariyoshi, et al., 1975; Williams, et al., 1975)

Chlorinated benzene	Free vicinal position	Possibility of epoxide formation	Extent of metabolism	P-450 n moles/g	ALA Synthetase n moles/ g/hr	Porphyria[a]
Control				0.68	22.6	
Monochlorobenzene	2,3,4,5,6	+	High	0.56	49.2	yes (1140)
1,2-Dichlorobenzene	3,4,5,6	+	High	0.66	36.3+	yes (455)
1,3-Dichlorobenzene	4,5,6	+	High	0.77	29.8+	-
1,4-Dichlorobenzene	2,3,5,6	+	High	0.73	32.3	yes (770)
1,2,3-Trichlorobenzene	4,5,6	+	High	0.84	33.6	yes (785)
1,2,4-Trichlorobenzene	5,6	+	High	1.63	47.5+	yes (730)
1,3,5-Trichlorobenzene	None	-	Low	0.73	34.8	-
1,2,3,4-Tetrachlorobenzene	5,6	+	High	0.97	33.3+	yes (660)
1,2,3,5-Tetrachlorobenzene	None	-	Low	1.14	35.4+	-
1,2,4,5-Tetrachlorobenzene	None	-	Low	0.99	27.5	-
Pentachlorobenzene	None	-	Very Low	1.16	98.7+	-
Hexachlorobenzene	None	-	Very Low	0.97	51.8	yes (400)

\+ Significantly increased over control.

[a] Numbers in parenthesis indicate dose in mg/kg body wt. used to produce porphyria in 5 days.

occurred. However, heme oxygenase activity tripled. Cytochrome P-450 content was not significantly different from control. At 100 and 200 mg/kg, Δ-ALA and heme oxygenase activities were increased further. At 100 mg/kg, cytochrome P-450 levels remained unchanged, but at 200 mg/kg, it was significantly lower than control ($p<0.005$).

Carlson and Tardiff (1976) studied the effects of chlorobenzene and other halogenated benzenes in adult male rats. All halogenated benzenes except chlorobenzene decreased hexobarbital sleeping time on the same day and/or 14 days following administration of the chemical. In addition, as can be seen in Table VII-7, CB induced decreases in cytochrome P-450 content, and increases in glucuronyl transferase levels.

Table VII-7

Effect of Chlorinated Benzenes Administered Orally for 14 Days on Various Parameters of Xenobiotic Metabolism
(Carlson and Tardiff, 1976)

Compound	Dose mg/kg/day)	Cytochrome c reductase (nmol cytochrome c reduced/min/ mg protein)*	Cytochrome p-450(E/mg protein x 10)	Glucuronyl-transferase (nmol/min/mg protein)	EPN+ detoxication (ug p-nitro-phenol/ 50 mg/30 min)	Benzpyrene hyroxylase (nmol/min/mg protein)	Azoreduc-tase (ng/min/m protein)
Chlorobenzene	0	103 ± 25[b]	236 ± 20[b]	5.8 ± 0.4[b]	6.7 ± 0.6[b]	1.39 ± 0.43[b]	
	200	110 ± 8[b]	209 ± 10[b]	9.4 ± 0.6[c]	7.1 ± 0.5[b]	0.89 ± 0.05[b]	
	400	109 ± 9[b]	197 ± 14[b]	12.2 ± 0.3[d]	8.4 ± 0.9[b]	0.92 ± 0.04[b]	
	800	78 ± 5[b]	133 ± 15[c]	13.0 ± 0.5[d]	7.4 ± 0.7[b]	0.78 ± 0.16[b]	
1,4-Dichloro-benzene	0	156 ± 9[b]	178 ± 20[b]	5.9 ± 0.6[b]	6.7 ± 0.6[b]	2.36 ± 0.49[b]	63.9 ± 2.8[b]
	10	151 ± 6[b]	174 ± 15[b]	7.8 ± 0.6[b,c]	7.2 ± 0.6[b,c]	1.75 ± 0.13[b]	67.5 ± 2.5[b,c]
	20	165 ± 8[b]	167 ± 11[b]	11.3 ± 1.0[d]	9.5 ± 1.1[c,d]	.53 ± 0.68[c]	71.4 ± 4.3[b,c]
	40	176 ± 10[b]	193 ± 13[b]	9.6 ± 0.6[c,d]	9.0 ± 0.5[d]	2.15 ± 0.34[b]	79.2 ± 6.6[c]
1-Bromo-4-chlorobenzene	0	149 ± 22[b]	205 ± 35[b]	5.8 ± 1.4[b]	9.5 ± 0.8[b]	1.51 ± 0.20[b]	59.1 ± 2.5[b]
	10	126 ± 6[b]	180 ± 7[b,c]	5.6 ± 0.8[b]	11.4 ± 0.9[b,c]	.37 ± 0.17[b]	60.9 ± 2.6[b,c]
	20	123 ± 4[b]	202 ± 7[b,c]	9.0 ± 0.7[c]	12.4 ± 1.1[c,d]	.06 ± 0.26[b,c]	69.7 ± 4.2[c]
	40	141 ± 5[b]	245 ± 19[c]	9.1 ± 0.5[c]	14.4 ± 0.6[d]	2.35 ± 0.23[c]	79.4 ± 2.7[d]
1,2,4-Trichloro-benzene	0	103 ± 8[b]	72 ± 13[b]	5.0 ± 0.4[b]	5.8 ± 0.3[b]	2.82 ± 0.53[b]	72.4 ± 11.1[b]
	10	139 ± 15[c]	99 ± 18[b]	5.1 ± 0.4[b]	10.3 ± 0.9[c]	3.82 ± 0.65[b]	102.0 ± 2.7[c]
	20	192 ± 12[d]	212 ± 45[c]	8.6 ± 1.6[c]	12.0 ± 0.8[c]	5.37 ± 1.01[b]	91.1 ± 4.6[c]
	40	183 ± 9[d]	268 ± 15[c]	8.3 ± 0.7[c]	15.4 ± 0.4[d]	5.22 ± 1.14[b]	130.7 ± 5.3[d]
Hexachloro-benzene	0	85 ± 7[b]	128 ± 18[b]	6.3 ± 1.0[b,c]	5.3 ± 0.9[b]	2.79 ± 0.54[b]	122.0 ± 24.9[b]
	10	106 ± 10[b]	204 ± 18[c]	4.5 ± 0.5[b]	15.0 ± 1.1[c]	4.76 ± 0.58[c]	187.7 ± 7.4[c]
	20	109 ± 7[b]	190 ± 16[c]	6.8 ± 0.3[c]	18.4 ± 0.8[c]	4.08 ± 0.37[b,c]	110.0 ± 7.4[c]
	40	100 ± 7[b]	150 ± 27[b,c]	6.6 ± 0.8[b,c]	18.9 ± 0.7[c]	4.24 ± 0.82[b,c]	262.7 ± 19.1[d]

* Value is mean ± S.E. for group of six rats except for benzpyrene hydroxylase group receiving 40 mg/kg of 1,2,4-trichlorobenzene. In that group, there were five rats. +EPN = O-ethyl O-nitrophenylphenylphosphorothioate

b-d Values which have been assigned the same superscript within each compound group do not differ significantly ($p>0.05$).

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the non-carcinogenic and carcinogenic effects. In the quantification of non-carcinogenic effects, an Adjusted Acceptable Daily Intake (AADI) for the chemical is determined. For ingestion data, this approach is illustrated as follows:

$$\text{Adjusted ADI} = \frac{(\text{NOAEL or MEL in mg/kg})(70\text{ kg})}{(\text{Uncertainty factor})(2\text{ liters/day})}$$

The 70 kg adult consuming 2 liters of water per day is used as the basis for the calculations. A "no-observed-adverse-effect-level" or a "minimal-effect-level" is determined from animal toxicity data or human effects data. This level is divided by an uncertainty factor because, for these numbers which are derived from animal studies, there is no universally acceptable quantitative method to extrapolate from animals to humans, and the possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human toxicity data, an uncertainty factor is used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxins. The guidelines set forth by the National Academy of Sciences (Drinking Water and Health, Vol. 1, 1977) are used in establishing uncertainty factors. These guidelines are as follows: an uncertainty factor of 10 is used if there exist valid experimental results on ingestion by humans, an uncertainty factor of 100 is used if there exist valid results on long-

term feeding studies on experimental animals, and an uncertainty factor of 1000 is used if only limited data are available.

In the quantification of carcinogenic effects, mathematical models are used to calculate the estimated excess cancer risks associated with the consumption of a chemical through the drinking water. EPA's Carcinogen Assessment Group has used the multistage model, which is linear at low doses and does not exhibit a threshold, to extrapolate from high dose animal studies to low doses of the chemical expected in the environment. This model estimates the upper bound (95% confidence limit) of the incremental excess cancer rate that would be projected at a specific exposure level for a 70 kg adult, consuming 2 liters of water per day, over a 70 year lifespan. Excess cancer risk rates also can be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. Current understanding of the biological mechanisms involved in cancer does not allow for choosing among the models. The estimates of incremental risks associated with exposure to low doses of potential carcinogens can differ by several orders of magnitude when these models are applied. The linear, non-threshold multi-stage model often gives one of the highest risk estimates per dose and thus would usually be the one most consistent with a regulatory philosophy which would avoid underestimating potential risk.

The scientific data base, which is used to support the estimating of risk rate levels as well as other scientific

endeavors, has an inherent uncertainty. In addition, in many areas, there exists only limited knowledge concerning the health effects of contaminants at levels found in drinking water. Thus, the dose-response data gathered at high levels of exposure are used for extrapolation to estimate responses at levels of exposure nearer to the range in which a standard might be set. In most cases, data exist only for animals; thus, uncertainty exists when the data are extrapolated to humans. When estimating risk rate levels, several other areas of uncertainty exist such as the effect of age, sex, species and target organ of the test animals used in the experiment, as well as the exposure mode and dosing rates. Additional uncertainty exists when there is exposure to more than one contaminant due to the lack of information about possible additive, synergistic or antagonistic interactions.

Non-Carcinogenic Effects

The principal toxic effects of chlorobenzene (CB) in humans and other animals include central nervous system (CNS) depression, blood dyscrasias, lung, liver and kidney damage. The appearance and intensity of these and other adverse effects are dependent upon dose and duration of exposure. Deaths in animals following high level acute exposure usually result from the CNS effects (primarily, respiratory failure). No human deaths have been reported following accidental exposure.

The oral LD_{50} for CB in rats and rabbits is about 3 g/kg (Irish, 1963; Vecerek, et al., 1976). Varshavskaya (1967)

determined oral LD_{50}s in several species in g/l. Conversion to dose/kg body weight was not possible from the available data. Nonetheless, the relative sensitivity of different species can be inferred (mouse = 1.44 g/l, rabbit = 2.25 g/l, rat = 2.39 g/l, guinea pig = 5.06 g/l). The inhalation LC_{50} in mice was determined by Bonnet et al.(1979) to be 8.2-9.1 mg/l and by Rozenbaum et al.(1947) to be 20 mg/l (2-hour exposure).

CB toxicity seen after non-fatal exposures is manifested principally as transient CNS effect (e.g., narcosis, somnolence, muscle spasms), blood dyscrasias (thrombocytopenia, leukopenia, lymphocytosis) and hepatic and renal toxicity. Reports of poisonings in humans described the victims as suffering from headaches, irritation of the eyes and respiratory passages, variable degrees of suppression of the level of consciousness, muscle spasms and dizziness (Reich, 1934; Rozenbaum, et al., 1947; Girard, et al., 1969; Smirnova and Granik, 1970). Rozenbaum, et al. (1947) reported transient thrombocytopenia and leukopenia in several exposed workers. They also observed leukocytosis in rabbits receiving a series of 2-20 0.9 mg/kg doses of CB over a 2-week period. In contrast, Cameron, et al.(1937) determined that CB, given subcutaneously or intravenously to rabbits, caused leukopenia when administered in daily doses of 1 ml for three successive days. Zub (1979) reported both phenomena in Swiss mice after inhalation exposure 7 hours/ day for three weeks at 2.5 mg/l or for three months at 0.1 mg/l. Mice and rats receiving 250, 500 or 750 mg/kg

CB in corn oil by gavage 5 times/week for 91 days exhibited significant histological changes including myeloid and lymphoid depletion and lymphoid necrosis (Battelle, 1978a,b). These effects were not seen at 60 or 125 mg/kg.

Another target organ of CB is the liver, in which the chemical causes necrosis and interferes with porphyrin metabolism, as do other chlorinated benzenes (Rimington and Ziegler, 1963; Varshavskaya, 1967; Khanin, 1969; Knapp, et al., 1971; Zub, 1979). CB also affects the kidney, in which congestive and necrotic changes are seen (Rozenbaum, et al., 1947; Irish, 1963; Khanin, 1969; Dilley, 1977).

Additional effects of CB which have been reported include alteration of pancreatic function (Yang, et al., 1979), pulmonary changes (Irish, 1963; Dilley, 1977) and emesis and abnormal stools (Monsanto, 1980).

1. One-day Health Advisory (1-Day HA)

No data are available from which to derive a 1-day HA for either the 10 kg child or the 70 kg adult. It is recommended that for this duration of exposure, the 10-day HA be applied (see below).

2. Ten-day Health Advisory (10-day HA)

No data developed via the oral route of exposure exist from which to derive a 10-day HA. The only appropriate data set comes from a teratology study in rats and rabbits (Hayes, et al., 1982). Animals were exposed to CB at levels of 0, 75, 210

or 590 ppm, 6 hr/day on days 6-15 (rats) or days 6-18 (rabbits) of pregnancy. No teratogenic effects were observed. Significant changes noted were increases in absolute and relative liver weights of rabbit dams exposed at 210 and 590 ppm. The NOAEL for this study was determined to be 75 ppm in the rabbits.

Using the data from the Hayes, et al study, a 10-day HA would be derived thusly:

$$TDI^{*} = 75 \text{ ppm} \times (4.6) \times 6 \text{ hr/day} \times 1 \text{ m}^3\text{/hr} \times 0.6 = 1242 \text{ mg/day}$$

$$TADI^{\dagger} = \frac{1242 \text{ mg/day}}{100 \times 70 \text{ kg}} = 0.18 \text{ mg/kg/day}$$

Where: 75 ppm = NOAEL, based upon lack of general toxicity in rabbits

4.6 = conversion factor from ppm to mg/m^3

6 hr/day = exposure duration

1 m^3/hr = estimated respiratory volume/hour in adult human

0.6 = assumed ratio of dose absorbed

100 = uncertainty factor, appropriate for use with NOAEL from animal data and no comparable human data

70 kg = assumed weight of average adult

(*TDI = Total Daily Intake in mg/day; †TADI = Total Allowable Daily Intake in mg/kg/day)

10-Day HA for the 10 kg child:

$$\frac{0.18 \text{ mg/kg/day} \times 10 \text{ kg}}{1 \text{ l}} = 1.8 \text{ mg/l}$$

Where: 0.18 mg/kg/day = TADI

10 kg = weight of protected individual

1 l = 1 liter, assumed volume of water consumed per day by 10 kg child

10-Day HA for the 70 kg adult:

$$\frac{0.18 \text{ mg/kg/day} \times 70 \text{ kg}}{2 \text{ l}} = 6.3 \text{ mg/l}$$

Where: 0.18 mg/kg/day = TADI

70 kg = weight of protected individual

2 l = 2 liters, assumed volume of water consumed per day by 70 kg adult

3. ADI (Acceptable Daily Intake)

A number of studies utilizing the oral route of exposure are available in which dose-response data are described. These studies identify no-observed-adverse-effect-levels (NOAELs) or lowest-adverse-effect-levels (LOAELs) which might be considered suitable for the derivation of an ADI.

The toxicity of chlorobenzene following oral administration was reported by Dow Chemical Company (Irish, 1963). CB was administered to rats five days/week for a total of 137 doses over 192 days, dose groups of 14.4, 144 or 288 mg/kg. In the middle and high dose groups, there were significant increases in liver and kidney weights and some "histopathological changes" in the liver. No significant changes were observed in the low dose group. Blood and bone marrow were normal in all animals.

The results of this study suggest an NOAEL of 14.4 mg/kg. An ADI could be derived thusly:

$$\frac{14.4 \text{ mg/kg/day} \times 70 \text{ kg} \times 5 \times 1.0}{100 \times 10 \times 7} = 0.72 \text{ mg/day}$$

Where: 14.4 mg/kg/day = NOAEL

70 kg = weight of protected individual

5/7 = conversion of 5 day/week exposure to 7 day/week exposure

1.0 = assumed ratio of dose absorbed

100 = uncertainty factor, appropriate for application to NOAEL in animal with no equivalent data in the human (longer-term exposure duration)

10 = uncertainty factor, appropriate for exposure duration significantly less than lifetime

Varshavskaya (1968) studied the effects of CB in white rats. The animals were treated daily with solutions of CB in doses of 0.001, 0.01 or 0.1 mg/kg using a stomach tube. Controls received sunflower oil alone. The experiments lasted nine months. A variety of effects were seen at the two higher doses. None were seen at the lowest dose. While this study suggests that effects can be seen at very low doses when compared with the other studies, little in the way of experimental detail was presented in her publication. Therefore, it is difficult to assess fully the results presented, and one cannot conclude that this paper should be the basis for the development of an ADI for CB.

Knapp, et al. (1971) described the subchronic toxicity of CB in rats as reported in Hazleton, 1967b. The animals were fed 12.5, 50, (100) or 250 mg/kg CB in their diets for 93-99 consecutive days. Male rats showed retarded growth at the

highest dose level. At the high dose level, a significant increase in liver and kidney weights was observed in females. However, no remarkable histological changes were noted. No effects were seen in the low dose group.

The results of this study suggest a NOAEL of 100 mg/kg/day. An acceptable daily intake (ADI) could be derived thusly:

$$\frac{100 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0}{100 \times 10} = 7.0 \text{ mg/day}$$

Where: 100 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = assumed ratio of dose absorbed

100 = uncertainty factor, appropriate for application to NOAEL in animal, with no equivalent data in the human (longer-term exposure duration)

10 = uncertainty factor, appropriate for exposure duration significantly less than lifetime

Subchronic studies were conducted in which both rats and mice were gavaged five times weekly with 0, 60, 125, 250, 500 or 750 mg/kg CB in corn oil (10 animals/species/sex/dose level) (Battelle, 1978c,d). Food consumption did not vary among the groups in mice, but it was lower in the two highest dose rat groups. Body weight gain was affected in both species, with significant changes observed in male mice at all doses. In rats, significant changes occurred only at the two highest doses. No clinically-significant CB-related changes were observed in any of the hematological parameters measured in either species. None of the clinical chemistry parameters measured in mice were changed. However, in the rats, SGPT and GGPT

levels were elevated in two surviving females and one male at 750 mg/kg. Urinalyses of the controls and two highest dose groups revealed a dose-dependent polyuria with concomitant decreases in specific gravity and creatinine concentration. Also, at the two highest doses, urinary coproporphyrin excretion was increased in both sexes. In mice, this increase was observed only in females at 250 and 500 mg/kg. Liver:body weight ratios were increased significantly in mice of both sexes at 500 and 750 mg/kg. A slight increase was seen in male mice at 125 mg/kg. Both male and female rats at 250 and 500 mg/kg showed an increase. Mice and rats at the three highest doses (250, 500 and 750 mg/kg) all exhibited significant histopathological changes including hepatic necrosis, nephrosis, myeloid depletion, lymphoid depletion and lymphoid necrosis.

From these data, a NOAEL of 125 mg/kg/day can be identified. An ADI could be developed thusly:

$$\frac{125 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 10 \times 7} = 6.0 \text{ mg/day}$$

Where: 125 mg/kg/day = NOAEL, based upon increased liver: body weight ratios at higher doses

70 kg = weight of protected individual

1.0 = assumed percentage of dose absorbed

5/7 = conversion of 5 day/week exposure to 7 day/week exposure

100 = uncertainty factor, appropriate for application to NOAEL in animal with no equivalent data in the human (longer-term exposure duration

10 = uncertainty factor, appropriate for exposure duration significantly less than lifetime

The only chronic exposure study of CB available is the NTP gavage bioassay in rats and mice (NTP, 1983). Both sexes of rats and female mice received 60 or 120 mg/kg/day in corn oil; male mice received 30 or 60 mg/kg/day. Doses were administered 5 days/week for up to 104 weeks.

Statistically-significant changes in non-carcinogenic end-points of toxicity included "equivocal mild to minimal liver necrosis" in the rats and a decrease in survival rate for low-dose male mice. The results of this study indicate that a NOAEL for rats of 60 mg/kg/day can be identified.

If the rat data were used, an ADI could be derived thusly:

$$\frac{60 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \quad x \quad 7} = 30.0 \text{ mg/day}$$

Where: 60 mg/kg/day = NOAEL from lifetime animal study

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of five day/week exposure regimen to seven day/week continuous exposure

100 = uncertainty factor, appropriate for use with animal data and equivalent duration of exposure

Existing Guidelines and Standards

[to be added]

Quantification of Non-carcinogenic Effects

The effects of CB in humans appear to be essentially the same as those observed in animal experiments. Several oral exposure animal studies are available in which acceptable dose-response data are described. The ADIs derived from these

Table VIII-1

Possible ADIs for Chlorobenzene

Experiment	Reference	Possible ADI
6-month inhalation (dogs)	Monsanto, 1980	2.0 mg/day
subchonic gavage (rats)	Irish, 1963	0.72 mg/day
subchronic gavage (rats)	Knapp, et al., 1971 (Hazleton, 1967b)	7.0 mg/day
subchronic gavage (rats and mice)	Battelle-Columbus, 1978c,d	6.0 mg/day
chronic gavage (rats and mice)	NTP, 1983	30.0 mg/day

studies range from 0.70 mg/day (Irish, 1963) to 30 mg/day (NTP, 1983) (see Table VIII-1).

The fact that the ADI generated from the chronic studies is greater than those derived from the subchronic studies suggests that the 10-fold uncertainty factor used to estimate an ADI from subchronic data may be unnecessarily large. As can be seen when comparing the bioassay prechronic studies (Battelle, 1978c,d) with the chronic studies (NTP, 1983), the high doses used in the chronic studies differed from those required to produce frank tissue injury in the subchronic studies by factors of 2-4. Yet, despite this relative closeness in doses, significant non-neoplastic lesions clearly attributable to chlorobenzene were not readily observed in the chronic study. On the other hand, one reason that the chronic study yielded

a larger ADI than did the studies of lesser duration exposure may be that the "special" parameters (i.e. urinalysis, clinical chemistry, hematology) were not included in the chronic study protocols. Had more sensitive indices of toxicity been evaluated in the chronic studies, effects may have been noted at one or more of the doses administered. In light of this consideration, it may not be appropriate to suggest that the 10-fold uncertainty factor is too large. In addition, the NTP Board of Scientific Counselors has not yet approved the report prepared on the bioassay of CB. Therefore, it is prudent to reserve judgment on its validity until such time as the report is approved.

At this time, then, the subchronic studies performed in the prechronic phase of the NTP bioassay provide the most appropriate data set from which to derive the ADI for chlorobenzene. If these data were used to develop an AADI, the AADI would be derived as follows:

$$\text{AADI} = \frac{\text{ADI}}{2\ 1} = \frac{6.0\ \text{mg/day}}{2\ 1} = 3.0\ \text{mg/l}$$

This AADI assumes that the protected individual (the 70 kg adult) drinks 2 liters of water/day and that the sole source of exposure to chlorobenzene is via that drinking water. [It is important to note that the taste and odor threshold in water has been identified at levels ranging from 0.41-1.5 ug/l (Tarkhova, 1965) to 10-20 ug/l (Varshavskaya, 1967). Therefore, any Maximum Contaminant Level (MCL) for chlorobenzene may have to consider the aesthetic, in addition to

the toxic, consequences following exposure to CB in drinking water].

Carcinogenic Effects

Chlorobenzene was tested for its carcinogenic potential in F344 rats and B6C3F1 mice in the NTP Bioassay Program. A draft report of the results of these studies is available (NTP, 1983). The experimental protocol is described on page VIII-11 of this document. With respect to carcinogenic responses, male rats dosed with CB exhibited a significant increase ($P<0.05$) in the incidence of animals with neoplastic nodules of the liver. There was no increase in incidence of hepatocelluar carcinomas in male rats or of neoplastic nodules or hepatocellular carcinomas in female rats. The draft report concludes that under the conditions of this bioassay, chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in high dose (120 mg/kg) male F344 rats, providing some but not clear evidence of carcinogenic activity of CB in male rats. Carcinogenic effects were not observed in female F344 rats or B6C3F1 mice of either sex.

Quantification of Carcinogenic Effects

The National Academy of Sciences, in Volume 5 of Drinking Water and Health, using a linearized, non-threshold multistage model, estimated a risk and an upper 95% confidence estimate of lifetime risk in humans following exposure to chlorobenzene (NAS, 1983). The Academy derived an estimate of risk to

exposure to a daily dose of 1 ug CB/liter of drinking water, based upon the limited evidence of carcinogenicity (liver neoplastic nodules in male rats). For a 70 kg adult drinking 2 liters of water per day, the estimated human lifetime risk at a daily dose of 1 ug/l = 3.71×10^{-8} and the upper 95% confidence estimate of lifetime cancer risk per ug/l = 2.13×10^{-7}.

Based upon the calculations, a range of CB concentrations that would be estimated to increase the risk by one excess cancer per million (10^6), per hundred thousand (10^5) and per ten thousand (10^4) persons over a 70-year lifetime can be extrapolated. These are summarized in Table VIII-2.

Recently, EPA classified chlorobenzene as to its carcinogenic potential, using the weight of evidence classification scheme proposed in its risk assessment guidelines for carcinogens (U.S. EPA, 1984). The Agency placed the chemical in Group C: Possibly carcinogenic to humans, but the Carcinogen Assessment Group did not to derive a carcinogenicity potency factor (q_1^*) or a range of excess lifetime cancer risk estimates. This option is available on a case-by-case basis for Group C chemicals. (Group C includes chemicals with limited evidence of carcinogenicity in animals in the absence of evidence in humans).

Table VIII-2

Drinking Water Concentrations and Estimated Excess Cancer Risk

Excess Lifetime Cancer Risk	Range of Concentrations (ug/l)[a]	
	NAS[b]	CAG[c]
10^{-4}	235	Not appropriate
10^{-5}	23.5	"
10^{-6}	2.35	"

[a] Assumes 2 liters of water consumed/day by 70 kg adult over a 70-year lifetime; number represents 95% upper bound confidence limit

[b] (NAS, 1983)

[c] The Carcinogen Assessment Group assessment concluded that, on the basis of the available data, no carcinogenic potency factor (q_1^*) or estimates of excess cancer risk would be derived for chlorobenzene

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